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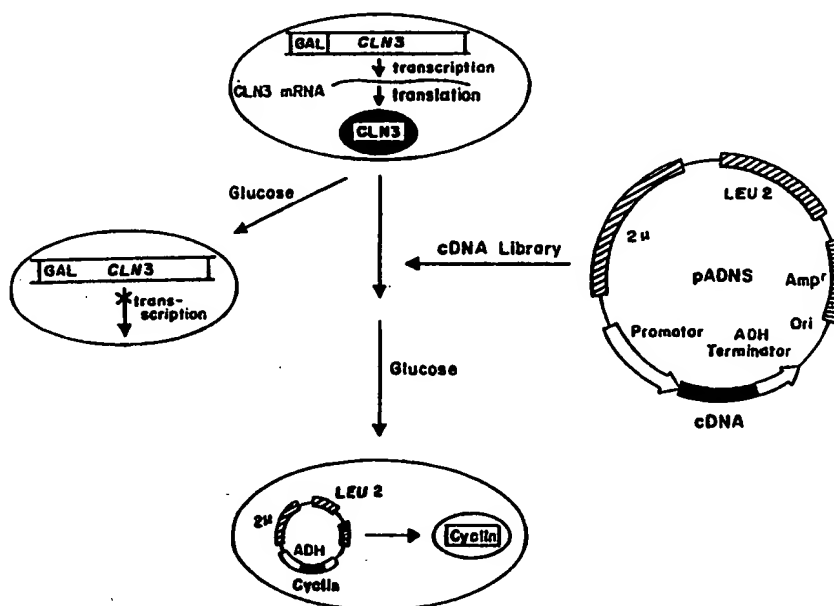
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**(54) Title:** D-TYPE CYCLIN AND USES RELATED THERETO



**(57) Abstract**

A novel class of cyclins is disclosed, referred to as D-type cyclins, of mammalian origin, particularly human origin. Also disclosed is: DNA and RNA encoding the novel cyclins; a method of identifying other D-type and non-D type cyclins; a method of detecting an increased level of a D-type cyclin and a method of inhibiting cell division by interfering with formation of the protein kinase-D type cyclin complex essential for cell cycle start.

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D-TYPE CYCLIN AND USES RELATED THERETODescriptionRelated Applications

This application is a continuation-in-part of United States  
5 Serial Number 07/701,514 filed May 16, 1991 and entitled "D-  
Type Cyclin and Uses Related Thereto" and also corresponds  
to and claims priority to Patent Cooperation Treaty  
Application (number not yet available) filed May 18, 1992  
and entitled "D-Type Cyclin and Uses Related Thereto." The  
10 teachings of U.S.S.N. 07/701,514 and the PCT Application  
filed May 18, 1992 are incorporated herein by reference.

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in the invention.

Background of the Invention

A typical cell cycle of a eukaryotic cell includes the M  
phase, which includes nuclear division (mitosis) and  
20 cytoplasmic division or cytokinesis and interphase, which  
begins with the G1 phase, proceeds into the S phase and ends  
with the G2 phase, which continues until mitosis begins,  
initiating the next M phase. In the S phase, DNA

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replication and histone synthesis occurs, while in the G1 and G2 phases, no net DNA synthesis occurs, although damaged DNA can be repaired. There are several key changes which occur during the cell cycle, including a critical point in the G1 phase called the restriction point or start, beyond which a cell is committed to completing the S, G2 and M phases.

Onset of the M phase appears to be regulated by a common mechanism in all eukaryotic cells. A key element of this mechanism is the protein kinase p34<sup>cdc2</sup>, whose activation requires changes in phosphorylation and interaction with proteins referred to as cyclins, which also have an ongoing role in the M phase after activation.

Cyclins are proteins that were discovered due to their intense synthesis following the fertilization of marine invertebrate eggs (Rosenthal, E.T. et al., Cell 20:487 (1980)). It was subsequently observed that the abundance of two types of cyclin, A and B, oscillated during the early cleavage divisions due to abrupt proteolytic degradation of the polypeptides at mitosis and thus, they derived their name (Evans, T. et al., Cell 33:389 (1983); Swenson, K.I. et al., Cell 47:867 (1986); Standart, N. et al., Dev. Biol. 124:248 (1987)).

Active rather than passive involvement of cyclins in regulation of cell division became apparent with the observation that a clam cyclin mRNA could cause activation of frog oocytes and entry of these cells into M phase (Swenson, K.I. et al., Cell 47:867 (1986)). Activation of frog oocytes is associated with elaboration of an M phase inducing factor known as MPF (Masui, Y. et al., J. Exp. Zool. 177:129 (1971); Smith, L.D. et al., Dev. Biol. 25:232 (1971)). MPF is a protein kinase in which the catalytic subunit is the frog homolog of the cdc2 protein kinase (Dunphy, W.G. et al., Cell 54:423 (1988); Gautier, J. et



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al., Cell 54:433 (1988); Arion, D. et al., Cell 55:371 (1988)).

Three types of classes of cyclins have been identified to date: B, A and CLN cyclins. The B-type cyclin has been  
5 shown to act in mitosis by serving as an integral subunit of the cdc2 protein kinase (Booher, R. et al. EMBO J. 6:3441 (1987); Draetta, G. et al., Cell 56:829 (1989); Labbe, J.C. et al., Cell 57:253 (1989); Labbe, J.C. et al., EMBO J. 8:3053 (1989); Meijer, L. et al., EMBO J. 8:2275 (1989);  
10 Cautier, J. et al., Cell 60:487 (1990)). The A-type cyclin also independently associates with the cdc2 kinase, forming an enzyme that appears to act earlier in the division cycle than mitosis (Draetta, G. et al., Cell 56:829 (1989); Minshull, J. et al., EMBO J. 9:2865 (1990); Giordano, A. et  
15 al., Cell 58:981 (1989); Pines, J. et al., Nature 346:760 (1990)). The functional difference between these two classes of cyclins is not yet fully understood.

Cellular and molecular studies of cyclins in invertebrate and vertebrate embryos have been accompanied by genetic  
20 studies, particularly in ascomycete yeasts. In the fission yeast, the cdc13 gene encodes a B-type cyclin that acts in cooperation with cdc2 to regulate entry into mitosis (Booher, R. et al., EMBO J. 6:3441 (1987); Booher, R. et al., EMBO J. 7:2321 (1988); Hagan, I. et al., J. Cell Sci.  
25 91:587 (1988); Solomon, M., Cell 54:738 (1988); Goebel, M. et al., Cell 54:433 (1988); Booher, R.N. et al., Cell 58:485 (1989)).

Genetic studies in both the budding yeast and fission yeast have revealed that cdc2 (or CDC28 in budding yeast) acts at  
30 two independent points in the cell cycle: mitosis and the so-called cell cycle "start" (Hartwell, L.H., J. Mol. Biol., 104:803 (1971); Nurse, P. et al., Nature 292:558 (1981); Piggot, J.R. et al., Nature 298:391 (1982); Reed, S.I. et al., Proc. Nat. Acad. Sci. USA 87:5697 (1990)).

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In budding yeast, the start function of the CDC28 protein also requires association of the catalytic subunit of the protein kinase with ancillary proteins that are structurally related to A and B- type cyclins. This third class of cyclin has been called the Cln class, and three genes comprising a partially redundant gene family have been described (Nash, R. et al., EMBO J. 7:4335 (1988); Hadwiger, J.A. et al., Proc. Natl. Acad. Sci. USA 86:6255 (1989); Richardson, H.E. et al., Cell 59:1127 (1989)). The CLN genes are essential for execution of start and in their absence, cells become arrested in the G1 phase of the cell cycle. The CLN1 and CLN2 transcripts oscillate in abundance through the cell cycle, but the CLN3 transcript does not. In addition, the Cln2 protein has been shown to oscillate in parallel with its mRNA (Nash, R. et al., EMBO J. 7:4335 (1988); Cross, F.R., Mol. Cell. Biol. 8:4675 (1988); Richardson, H.E. et al., Cell 59:1127 (1988); Wittenberg, et al., 1990)).

Although the precise biochemical properties conferred on cdc2/CDC28 by association with different cyclins have not been fully elaborated, genetic studies of cyclin mutants clearly establishes that they confer "G1" and "G2" properties on the catalytic subunit (Booher, R. and D. Beach, EMBO J. 6:3441 (1987); Nash, R. et al., EMBO J. 7:4335 (1988); Richardson, H.E. et al., Cell 56:1127 (1989)).

cdc2 and cyclins have been found not only in embryos and yeasts, but also in somatic human cells. The function of the cdc2/cyclin B enzyme appears to be the same in human cells as in other cell types (Riabowol, K. et al., Cell 57:393 (1989)). A human A type cyclin has also been found in association with cdc2. No CLN type cyclin has yet been described in mammalian cells. A better understanding of the elements involved in cell cycle regulation and of their interactions would contribute to a better understanding of

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cell replication and perhaps even alter or control the process.

### Summary of the Invention

The present invention relates to a novel class of cyclins, referred to as D-type cyclins, which are of mammalian origin and are a new family of cyclins related to, but distinct from, previously described A, B or CLN type cyclins. In particular, it relates to human cyclins, encoded by genes shown to be able to replace a CLN-type gene essential for cell cycle start in yeast, which complement a deficiency of a protein essential for cell cycle start and which, on the basis of protein structure, are on a different branch of the evolutionary tree from A, B or CLN type cyclins. Three members of the new family of D-type cyclins, referred to as the human D-type gene family, are described herein. They encode small (33-34 KDa) proteins which share an average of 57% identity over the entire coding region and 78% in the cyclin box. One member of this new cyclin family, cyclin D1 or CCND1, is 295 amino acid residues and has an estimated molecular weight of 33,670 daltons (Da). A second member, cyclin D2 or CCND2, is 289 amino acid residues and has an estimated molecular weight of 33,045 daltons. It has been mapped to chromosome 12p band p13. A third member, cyclin D3 or CCND3, is 292 amino acid residues and has an estimated molecular weight of approximately 32,482 daltons. It has been mapped to chromosome 6p band p21. The D-type cyclins described herein are the smallest cyclin proteins identified to date. All three cyclin genes described herein are interrupted by an intron at the same position. D-type cyclins of the present invention can be produced using recombinant techniques, can be synthesized chemically or can be isolated or purified from sources in which they occur naturally. Thus, the present invention includes recombinant D-type cyclins, isolated or purified D-type cyclins and synthetic D-type cyclins.

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The present invention also relates to DNA or RNA encoding a D-type cyclin of mammalian origin, particularly of human origin, as well as to antibodies, both polyclonal and monoclonal, specific for a D-type cyclin of mammalian, particularly human, origin.

The present invention further relates to a method of isolating genes encoding other cyclins, such as other D-type cyclins and related (but non-D type) cyclins. It also has diagnostic and therapeutic aspects. For example, it relates to a method in which the presence and/or quantity of a D-type cyclin (or cyclins) in tissues or biological samples, such as blood, urine, feces, mucous or saliva, is determined, using a nucleic acid probe based on a D-type cyclin gene or genes described herein or an antibody specific for a D-type cyclin. This embodiment can be used to predict whether cells are likely to undergo cell division at an abnormally high rate (i.e. if cells are likely to be cancerous), by determining whether their cyclin levels or activity are elevated (elevated level of activity being indicative of an increased probability that cells will undergo an abnormally high rate of division). The present method also relates to a diagnostic method in which the occurrence of cell division at an abnormally high rate is assessed based on abnormally high levels of a D-type cyclin(s), a gene(s) encoding a D-type cyclin(s) or a transcription product(s) (RNA).

In addition, the present invention relates to a method of modulating (decreasing or enhancing) cell division by altering the activity of at least one D-type cyclin, such as D2, D2 or D3 in cells. The present invention particularly relates to a method of inhibiting increased cell division by interfering with the activity or function of a D-type cyclin(s). In this therapeutic method, function of D-type cyclin(s) is blocked (totally or partially) by interfering with its ability to activate the protein kinase it would otherwise (normally) activate (e. g., p34<sup>cdc2</sup> or a related

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protein kinase), by means of agents which interfere with D-type cyclin activity, either directly or indirectly. Such agents include anti-sense sequences or other transcriptional modulators which bind D cyclin-encoding DNA or RNA; antibodies which bind either the D-type cyclin or a molecule with which a D-type cyclin must interact or bind in order to carry out its role in cell cycle start; substances which bind the D-type cyclin(s); agents (e.g. proteases) which degrade or otherwise inactivate the D-type cyclin(s); or agents (e.g., small organic molecules) which interfere with association of the D-type cyclin with the catalytic subunit of the kinase. The subject invention also relates to agents (e. g., oligonucleotides, antibodies, peptides) useful in the isolation, diagnostic or therapeutic methods described.

15 Brief Description of the Figures

Figure 1 is a schematic representation of a genetic screen for human cyclin genes.

Figure 2 is the human cyclin D1 nucleic acid sequence (SEQ ID No. 1) and amino acid sequence (SEQ ID No. 2), in which nucleotide numbers and amino acid numbers are on the right, amino acid numbers are given with the initiation methionine as number one and the stop codon is indicated by an asterisk.

Figure 3 is the human cyclin D2 nucleic acid sequence (SEQ ID No. 3) and amino acid sequence (SEQ ID No. 4) in which nucleotide numbers and amino acid numbers are on the right, amino acid numbers are given with the initiation methionine as number one and the stop codon is indicated by an asterisk.

Figure 4 is the human cyclin D3 nucleic acid sequence (SEQ ID No. 5) and amino acid sequence (SEQ ID No. 6), in which nucleotide numbers and amino acid numbers are on the right, amino acid numbers are given with the initiation methionine

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as number one and the stop codon is indicated by an asterisk.

Figure 5 shows the cyclin gene family.

Figure 5A shows the amino acid sequence alignment of seven  
5 cyclin genes (CYCD1-Hs, SEQ ID No. 7; CYCA-Hs, SEQ ID No. 8;  
CYCA-Dm, SEQ ID No. 9; CYCB1-Hs, SEQ ID No. 10; CDCl3-Sp,  
SEQ ID No. 11; CLN1-Sc, SEQ ID No. 12; CLN3-Sc, SEQ ID No.  
13), in which numbers within certain sequences indicate the  
number of amino acid residues omitted from the sequence as  
10 the result of insertion.

Figure 5B is a schematic representation of the evolutionary  
tree of the cyclin family, constructed using the Neighbor-  
Joining method; the length of horizontal line reflects the  
divergence.

15 Figure 6 shows alternative polyadenylation of the cyclin D1  
gene transcript.

Figure 6A is a comparison of several cDNA clones isolated  
from different cell lines. Open boxes represent the 1.7 kb  
small transcript containing the coding region of cyclin D1  
20 gene. Shadowed boxes represent the 3' fragment present in  
the 4.8 kb long transcript. Restriction sites are given  
above each cDNA clone to indicate the alignment of these  
clones.

Figure 6B shows the nucleotide sequence surrounding the  
25 first polyadenylation site for several cDNA clones (CYCD1-  
21, SEQ ID No. 14; CYCD1-H12, SEQ ID No. 15; CYCD1-HO34, SEQ  
ID No. 16; CYCD1-TO78, SEQ ID No. 17 and a genomic clone;  
CYCD1-GO68, SEQ ID No. 18).

Figure 6C is a summary of the structure and alternative  
30 polyadenylation of the cyclin D1 gene. Open boxes represent  
the small transcript, the shadowed box represents the 3'

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sequence in the large transcript and the filled boxes indicate the coding regions.

Figure 7 shows the protein sequence comparison of eleven mammalian cyclins (CYCD1-Hs, SEQ ID No. 19; CYL1-Mm, SEQ ID  
5 No. 20; CYCD2-Hs, SEQ ID No. 21; CYCL2-Mm, SEQ ID No. 22; CYCD3-Hs, SEQ ID No. 23; CYL3-Mm, SEQ ID No. 24; CYCA-Hs, SEQ ID No. 25; CYCB1-Hs, SEQ ID No. 26; CYCB2-Hs, SEQ ID No. 27; CYGC-Hs, SEQ ID No. 28; CYCE-Hs, SEQ ID No. 29).

Figure 8 is a schematic representation of the genomic  
10 structure of human cyclin D genes, in which each diagram represents one restriction fragment from each cyclin D gene that has been completely sequenced. Solid boxes indicate exon sequences, open boxes indicate intron or 5' and 3' untranslated sequences and hatched boxes represent  
15 pseudogenes. The positions of certain restriction sites, ATG and stop codons are indicated at the top of each clone.

Figure 9 is the nucleic acid sequence (SEQ ID No. 30) and amino acid sequence (SEQ ID No. 31) of a cyclin D2 pseudogene.

20 Figure 10 is the nucleic acid sequence (SEQ ID No. 32) and the amino acid sequence (SEQ ID No. 33) of a cyclin D3 pseudogene.

Figure 11 is the nucleic acid sequence (SEQ ID No. 34) of 1.3 kb of human cyclin D1 promoter; the sequence ends at  
25 initiation ATG codon and transcript ion starts at approximately nucleotide -160.

Figure 12 is the nucleotide sequence (SEQ ID No. 35) of 1.6 kb of human cyclin D2 promoter; the sequence ends at initiation ATG codon and transcript ion starts at  
30 approximately nucleotide -170.

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Figure 13 is the nucleotide sequence (SEQ ID No. 36) of 3.2 kb of human cyclin D3 promoter; the sequence ends at initiation ATG codon and transcription starts at approximately nucleotide -160.

## 5 Detailed Description of the Invention

As described herein, a new class of mammalian cyclin proteins, designated D-type cyclins, has been identified, isolated and shown to serve as a control element for the cell cycle start, in that they fill the role of a known cyclin protein by activating a protein kinase whose activation is essential for cell cycle start, an event in the G1 phase at which a cell becomes committed to cell division. Specifically, human D-type cyclin proteins, as well as the genes which encode them, have been identified, isolated and shown to be able to replace CLN type cyclin known to be essential for cell cycle start in yeast. The chromosomal locations of CCND2 and CCND3 have also been mapped.

As a result, a new class of cyclins (D type) is available, as are DNA and RNA encoding the novel D-type cyclins, antibodies specific for (which bind to) D-type cyclins and methods of their use in the identification of additional cyclins, the detection of such proteins and oligonucleotides in biological samples, the inhibition of abnormally increased rates of cell division and the identification of inhibitors of cyclins.

The following is a description of the identification and characterization of human D-type cyclins and of the uses of these novel cyclins and related products.

## 30 Isolation and Characterization of Human Cyclin D1, D2 and D3

As represented schematically in Figure 1 and described in detail in Example 1, a mutant yeast strain in which two of



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the three CLN genes (CLN1 and CLN2) were inactive and expression of the third was conditional, was used to identify human cDNA clones which rescue yeast from CLN deficiency. A human glioblastoma cDNA library carried in a yeast expression vector (pADNS) was introduced into the mutant yeast strain. Two yeast transformants (pCYCD1-21 and pCYCD1-19) which grew despite the lack of function of all three CLN genes and were not revertants, were identified and recovered in E. coli. Both rescued the mutant (CLN deficient) strain when reintroduced into yeast, although rescue was inefficient and the rescued strain grew relatively poorly.

pCYCD1-19 and pCYCD1-21 were shown, by restriction mapping and partial DNA sequence analysis, to be independent clones representing the same gene. A HeLa cDNA library was screened for a full length cDNA clone, using the 1.2 kb insert of pCYCD1-21 as probe. Complete sequencing was done of the longest of nine positive clones identified in this manner (pCYCD1-H12; 1325 bp). The sequence of the 1.2 kb insert is presented in Figure 2; the predicted protein product of the gene is of approximate molecular weight 34,000 daltons.

Cyclin D2 and cyclin D3 cDNAs were isolated using the polymerase chain reaction and three oligonucleotide probes derived from three highly conserved regions of D-type cyclins, as described in Example 4. As described, two 5' oligonucleotides and one 3' degenerate oligonucleotide were used for this purpose. The nucleotide and amino acid sequences of the CCND2 gene and encoded D2 cyclin protein are represented in Figure 3 and of the CCND3 gene and encoded D3 cyclin protein are represented in Figure 4. A deposit of plasmid pCYC-D3 was made with the American Type Culture Collection (Rockville, MD) on May 14, 1991, under the terms of the Budapest Treaty. Accession number 68620 has been assigned to the deposit.

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Comparison of the CYCD1-H12-encoded protein sequence with that of known cyclins (see Figure 5A) showed that there was homology between the new cyclin and A, B and CLN type cyclins, but also made it clear that CYCD1 differs from these existing classes.

An assessment of how this new cyclin gene and its product might be related in an evolutionary sense to other cyclin genes was carried out by a comprehensive comparison of the amino acid sequences of all known cyclins (Figure 5B and Example 1). Results of this comparison showed that CYCD1 represents a new class of cyclin, designated herein cyclin D.

Expression of cyclin D1 gene in human cells was studied using Northern analysis, as described in Example 2. Results showed that levels of cyclin D1 expression were very low in several cell lines. The entire coding region of the CYCD1 gene was used to probe poly(A)+ RNA from HeLa cells and demonstrated the presence of two major transcripts, one approximately 4.8 kb and the other approximately 1.7 kb, with the higher molecular weight form being the more abundant. Most of the cDNA clones isolated from various cDNA libraries proved to be very similar to clone \_CYCD1-H12 and, thus, it appears that the 1.7 kb transcript detected in Northern blots corresponds to the nucleotide sequence of Figure 2. The origin of the larger (4.8 kb) transcript was unclear. As described in Example 2, it appears that the two mRNAs detected (4.8 kb and 1.7 kb) arose by differential polyadenylation of CYCD1 (Figure 6).

Differential expression of cyclin D1 in different tissues and cell lines was also assessed, as described in Example 3. Screening of cDNA libraries to obtain full length CYCD1 clones had demonstrated that the cDNA library from the human glioblastoma cell line (U118 MG) used to produce yeast transformants produced many more positives than the other three cDNA libraries (human HeLa cell cDNA, human T cell

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cDNA, human teratocarcinoma cell cDNA). Northern and Western blotting were carried out to determine whether cyclin D1 is differentially expressed. Results showed (Example 3) that the level of transcript is 7 to 10 fold  
5 higher in the glioblastoma (U118 MG) cells than in HeLa cells, and that in both HeLa and U118 MG cells, the high and low molecular weight transcripts occurred. Western blotting using anti-CYL1 antibody readily detected the presence of a 34kd polypeptide in the glioblastoma cells and demonstrated  
10 that the protein is far less abundant in HeLa cells and not detectable in the 293 cells. The molecular weight of the anti-CYCL1 cross reactive material identified in U118 MG and HeLa cells is exactly that of the human CYCD1 protein expressed in E. coli. Thus, results demonstrated  
15 differential occurrence of the cyclin D1 in the cell types analyzed, with the highest levels being in cells of neural origin.

As also described herein (Example 6), human genomic libraries were screened using cDNA probes and genomic clones  
20 of human D-type cyclins, specifically D1, D2 and D3, have been isolated and characterized. Nucleic acid sequences of cyclin D1, D2 and D3 promoters are represented in Figures 11-13. Specifically, the entire 1.3 kb cyclin D1 cDNA clone was used as a probe to screen a normal human liver genomic  
25 library, resulting in identification of three positive clones. One of these clones (G6) contained a DNA insert shown to contain 1150 bp of upstream promoter sequence and a 198 bp exon, followed by an intron. Lambda genomic clones corresponding to the human cyclin D2 and lambda genomic  
30 clones corresponding to the human cyclin D3 were also isolated and characterized, using a similar approach. One clone ( $\lambda$ D2-G4) was shown to contain (Figure 8B) a 2.7 kb SacI SmaI fragment which includes 1620 bp of sequence 5' to the presumptive initiating methionine codon identified in D2  
35 cDNA (Figure 3) and a 195 bp exon followed by a 907 bp intervening sequence. One clone (G9) was shown to contain (Figure 8C) 1.8 kb of sequence 5' to the presumptive

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initiating methionine codon identified in D3 cDNA (Figure 4), a 198 bp exon 1, a 684 bp exon 2 and a 870 bp intron.

Thus, as a result of the work described herein, a novel class of mammalian cyclins, designated cyclin D or D-type cyclin, has been identified and shown to be distinct, on the basis of structure of the gene (protein) product, from previously-identified cyclins. Three members of this new class, designated cyclin D1 or CCND1, cyclin D2 or CCND2 and cyclin D3 or CCND3, have been isolated and sequenced. They have been shown to fulfill the role of another cyclin (CLN type) in activation of the protein kinase (CDC28) which is essential for cell cycle start in yeast. It has also been shown that the cyclin D1 gene is expressed differentially in different cell types, with expression being highest in cells of neural origin.

#### Uses of the Invention

It is possible, using the methods and materials described herein, to identify genes (DNA or RNA) which encode other cyclins (DNA or RNA which replaces a gene essential for cell cycle start). This method can be used to identify additional members of the cyclin D class or other (non-D type) cyclins of either human or nonhuman origin. This can be done, for example, by screening other cDNA libraries using the budding yeast strain conditional for CLN cyclin expression, described in Example 1, or another mutant in which the ability of a gene to replace cyclin expression can be assessed and used to identify cyclin homologues. This method is carried out as described herein, particularly in Example 1 and as represented in Figure 1. A cDNA library carried in an appropriate yeast vector (e.g., pADNS) is introduced into a mutant yeast strain, such as the strain described herein (Example 1 and Experimental Procedures). The strain used contains altered CLN genes. In the case of the specific strain described herein, insertional mutations in the CLN1 and CLN2 genes rendered them inactive and

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alteration of the CLN3 gene allowed for its conditional expression from a galactose-inducible, glucose-repressible promoter; as exemplified, this promoter is a galactose-inducible, glucose-repressible promoter but others can be used.

Mutant yeast transformed with the cDNA library in the expression vector are screened for their ability to grow on glucose-containing medium. In medium containing galactose, the CLN3 gene is expressed and cell viability is maintained, despite the absence of CLN1 and CLN2. In medium containing glucose, all CLN function is lost and the yeast cells arrest in the G1 phase of the cell cycle. Thus, the ability of a yeast transformant to grow on glucose-containing medium is an indication of the presence in the transformant of DNA able to replace the function of a gene essential for cell cycle start. Although not required, this can be confirmed by use of an expression vector, such as pADNS, which contains a selectable marker (the LEU2 marker is present in pADNS). Assessment of the plasmid stability shows whether the ability to grow on glucose-containing medium is the result of reversion or the presence of DNA function (introduction of DNA which replaces the unexpressed or nonfunctional yeast gene(s) essential for cell cycle start). Using this method, cyclins of all types (D type, non-D type) can be identified by their ability to replace CLN3 function when transformants are grown on glucose.

Screening of additional cDNA or genomic libraries to identify other cyclin genes can be carried out using all or a portion of the human D-type cyclin DNAs disclosed here in as probes; for example, all or a portion of the D1, D2 or D3 cDNA sequences of Figures 2-4, respectively, or all or a portion of the corresponding genomic sequences described herein can be used as probes. The hybridization conditions can be varied as desired and, as a result, the sequences identified will be of greater or lesser complementarity to the probe sequence (i.e., if higher or lower stringency

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conditions are used). Additionally, an anti-D type cyclin antibody, such as CYL1 or another raised against D1 or D3 or other human D-type cyclin, can be used to detect other recombinant D-type cyclins produced in appropriate host  
5 cells transformed with a vector containing DNA thought to encode a cyclin.

Based on work described herein, it is possible to detect altered expression of a D-type cyclin or increased rates of cell division in cells obtained from a tissue or biological  
10 sample, such as blood, urine, feces, mucous or saliva. This has potential for use for diagnostic and prognostic purposes since, for example, there appears to be a link between alteration of a cyclin gene expression and cellular transformation or abnormal cell proliferation. For example,  
15 several previous reports have suggested the oncogenic potential of altered human cyclin A function. The human cyclin A gene was found to be a target for hepatitis B virus integration in a hepato-cellular carcinoma (Wand, J. et al., Nature 343:555 (1990)). Cyclin A has also been shown to  
20 associate with adenovirus E1A in virally infected cells (Giordano, A. et al., Cell 58:981 (1989); Pines, J. et al., Nature 346:760 (1990)). Further, the PRAD1 gene, which has the same sequence as the cyclin D1 gene, may play an important role in the development of various tumors (e.g.,  
25 non-parathyroid neoplasia, human breast carcinomas and squamous cell carcinomas) with abnormalities in chromosome 11q13. In particular, identification of CCND1 (PRAD1) as a candidate BCL1 oncogene provides the most direct evidence for the oncogenic potential of cyclin genes. This also  
30 suggests that other members of the D-type cyclin family may be involved in oncogenesis. In this context, the chromosomal locations of the CCND2 and CCND3 genes have been mapped to 12p13 and 6p21, respectively. Region 12p13 contains sites of several translocations that are associated  
35 with specific immunophenotypes of disease, such as acute lymphoblastic leukemia, chronic myelomonocytic leukemia, and acute myeloid leukemia. Particularly, the isochromosome of

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the short arm of chromosome 12 [1(12p)] is one of a few known consistent chromosomal abnormalities in human solid tumors and is seen in 90% of adult testicular germ cell tumors. Region 6p21, on the other hand, has been implicated  
5 in the manifestation of chronic lymphoproliferative disorder and leiomyoma. Region tp21, the locus of HLA complex, is also one of the best characterized regions of the human genome. Many diseases have been previously linked to the KLA complex, but the etiology of few of these diseases is  
10 fully understood. Molecular cloning and chromosomal localization of cyclins D2 and D3 should make it possible to determine whether they are directly involved in these translocations, and if so, whether they are activated. If they prove to be involved, diagnostic and therapeutic  
15 methods described here in can be used to assess an individual's disease state or probability of developing a condition associated with or caused by such translocations, to monitor therapy effectiveness (by assessing the effect of a drug or drugs on cell proliferation) and to provide  
20 treatment.

The present invention includes a diagnostic method to detect altered expression of a cyclin gene, such as cyclin D1, D2, D3 or another D-type cyclin. The method can be carried out to detect altered expression in cells or in a biological  
25 sample. As shown herein, there is high sequence similarity among cyclin D genes, which indicates that different members of D-type cyclins may use similar mechanisms in regulating the cell cycle (e.g., association with the same catalytic subunit and acting upon the same substrates). The fact that  
30 there is cell-type-specific differential expression, in both mouse and human cells, makes it reasonable to suggest that different cell lineages or different tissues may use different D-type cyclins to perform very similar functions and that altered tissue-specific expression of cyclin D  
35 genes as a result of translocation or other mutational events may contribute to abnormal cell proliferation. As described herein, cyclin D1 is expressed differentially in

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tissues analyzed; in particular, it has been shown to be expressed at the highest levels in cells of neural origin (e.g., glioblastoma cells).

As a result of the work described herein, D-type cyclin  
5 expression can be detected and/or quantitated and results used as an indicator of normal or abnormal (e.g., abnormally high rate of) cell division. Differential expression (either expression in various cell types or of one or more of the types of D cyclins) can also be determined.

- 10 In a diagnostic method of the present invention, cells obtained from an individual are processed in order to render nucleic acid sequences in them available for hybridization with complementary nucleic acid sequences. All or a portion of the D1, D2 and/or D3 cyclin (or other D-type cyclin gene)  
15 sequences can be used as a probe(s). Such probes can be a portion of a D-type cyclin gene; such a portion must be of sufficient length to hybridize to complementary sequences in a sample and remain hybridized under the conditions used and will generally be at least six nucleotides long.  
20 Hybridization is detected using known techniques (e.g., measurement of labeled hybridization complexes, if radiolabeled or fluorescently labeled oligonucleotide probed are used). The extent to which hybridization occurs is quantitated; increased levels of the D-type cyclin gene is  
25 indicative of increased potential for cell division.

Alternatively, the extent to which a D-type cyclin (or cyclins) is present in cells, in a specific cell type or in a body fluid can be determined using known techniques and an antibody specific for the D-type cyclin(s). In a third type  
30 of diagnostic method, complex formation between the D-type cyclin and the protein kinase with which it normally or typically complexes is assessed, using exogenous substrate, such as histone H1, as a substrate. Arion, D. et al., Cell, 55:371 (1988). In each diagnostic method, comparison of  
35 results obtained from cells or a body fluid being analyzed



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with results obtained from an appropriate control (e.g., cells of the same type known to have normal D-type cyclin levels and/or activity or the same body fluid obtained from an individual known to have normal D-type cyclin levels and/or activity) is carried out. Increased D-type cyclin levels and/or activity may be indicative of an increased probability of abnormal cell proliferation or oncogenesis or of the actual occurrence of abnormal proliferation or oncogenesis. It is also possible to detect more than one type of cyclin (e.g., A, B, and/or D) in a cell or tissue sample by using a set of probes (e.g., a set of nucleic acid probes or a set of antibodies), the members of which each recognize and bind to a selected cyclin and collectively provide information about two or more cyclins in the tissues or cells analyzed. Such probes are also the subject of the present invention; they will generally be detectably labelled (e.g., with a radioactive label, a fluorescent material, biotin or another member of a binding pair or an enzyme).

A method of inhibiting cell division, particularly cell division which would otherwise occur at an abnormally high rate, is also possible. For example, increased cell division is reduced or prevented by introducing into cells a drug or other agent which can block, directly or indirectly, formation of the protein kinase-D type cyclin complex and, thus, block activation of the enzyme. In one embodiment, complex formation is prevented in an indirect manner, such as by preventing transcription and/or translation of the D-type cyclin DNA and/or RNA. This can be carried out by introducing antisense oligonucleotides into cells, in which they hybridize to the cyclin-encoding nucleic acid sequences, preventing their further processing. It is also possible to inhibit expression of the cyclin by interfering with an essential D-type transcription factor.

There are reasons to believe that the regulation of cyclin gene transcription may play an important role in regulating the cell cycle and cell growth and oscillations of cyclin

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mRNA levels are critical in controlling cell division. The G1 phase is the time at which cells commit to a new round of division in response to external and internal sequences and, thus, transcription factors which regulate expression of G1 cyclins are surely important in controlling cell proliferation. Modulation of the transcription factors is one route by which D-type cyclin activity can be influenced, resulting, in the case of inhibition or prevention of function of the transcription factor(s), in reduced D-type cyclin activity. Alternatively, complex formation can be prevented indirectly by degrading the D-type cyclin(s), such as by introducing a protease or substance which enhances cyclin breakdown into cells. In either case, the effect is indirect in that less D-type cyclin is available than would otherwise be the case.

In another embodiment, protein kinase-D type cyclin complex formation is prevented in a more direct manner by, for example, introducing into cells a drug or other agent which binds the protein kinase or the D-type cyclin or otherwise interferes with the physical association between the cyclin and the protein kinase it activates (e.g., by intercalation) or disrupts the catalytic activity of the enzyme. This can be effected by means of antibodies which bind the kinase or the cyclin or a peptide or low molecular weight organic compound which, like the endogenous D-type cyclin, binds the protein kinase, but whose binding does not result in activation of the enzyme or results in its being disabled or degraded. Peptides and small organic compounds to be used for this purpose can be designed, based on analysis of the amino acid sequences of D-type cyclins, to include residues necessary for binding and to exclude residues whose presence results in activation. This can be done, for example, by systematically mapping the binding site(s) and designing molecules which recognize or otherwise associate with the site(s) necessary for activation, but do not cause activation. As described herein, there is differential expression in tissues of D-type cyclins. Thus, it is

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possible to selectively decrease mitotic capability of cells by the use of an agent (e.g., an antibody or anti-sense or other nucleic acid molecule) which is designed to interfere with (inhibit) the activity and/or level of expression of a selected type (or types) of D cyclin. For example, in treating tumors involving the central nervous system or other non-hematopoietic tissues, agents which selectively inhibit cyclin D1 might be expected to be particularly useful, since D1 has been shown to be differentially expressed (expressed at particularly high levels in cells of neural origin).

Antibodies specifically reactive with D-type cyclins of the present invention can also be produced, using known methods. For example, anti-D type cyclin antisera can be produced by injecting an appropriate host (e.g. rabbits, mice, rats, pigs) with the D-type cyclin against which anti sera is desired and withdrawing blood from the host animal after sufficient time for antibodies to have been formed. Monoclonal antibodies can also be produced using known techniques. Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).

The present invention also includes a method of screening compounds or molecules for their ability to inhibit or suppress the function of a cyclin, particularly a D-type cyclin. For example, mutant cells as described herein, in which a D-type cyclin such as D1 or D3, is expressed, can be used. A compound or molecule to be assessed for its ability to inhibit a D-type cyclin is contacted with the cells, under conditions appropriate for entry of the compound or molecule into the cells. Inhibition of the cyclin will result in arrest of the cells or a reduced rate of cell division. Comparison of the rate or extent of cell division in the presence of the compound or molecule being assessed with cell division of an appropriate control (e.g. the same type of cells without added test drug) will

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demonstrate the ability or inability of the compound or molecule to inhibit the cyclin. Existing compounds or molecules (e.g., those present in a fermentation broth or a chemical "library") or those developed to inhibit the cyclin activation of its protein kinase can be screened for their effectiveness using this method. Drugs which inhibit D-type cyclin are also the subject of this invention.

The present invention will now be illustrated by the following examples, which are not intended to be limiting in any way.

### EXAMPLES

Experimental procedures for Examples 1-3 are presented after Example 3.

EXAMPLE 1:            Identification of Human cDNA Clones That Rescue CLN Deficiency

In S. cerevisiae, there are three Cln proteins. Disruption of any one CLN gene has little effect on growth, but if all three CLN genes are disrupted, the cells arrest in G1 (Richardson, H.E. et al., Cell 59:1127 (1989)). A yeast strain was constructed, as described below, which contained insertional mutations in the CLN1 and CLN2 genes to render them inactive. The remaining CLN3 gene was further altered to allow for conditional expression from the galactose-inducible glucose-repressible promoter GAL1 (see Figure 1). The strain is designated 305-15d #21. In medium containing galactose, the CLN3 gene is expressed and despite the absence of both CLN1 and CLN2, cell viability is retained (Figure 1). In a medium containing glucose, all CLN function is lost and the cells arrest in the G1 phase of the cell cycle.

A human glioblastoma cDNA library carried in the yeast expression vector pADNS (Colicelli, J. et al., Pro. Natl. Acad. Sci. USA 86:3599 (1989)) was introduced into the

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yeast. The vector pADNS has the LEU2 marker, the 2 $\mu$  replication origin, and the promoter and terminator sequences from the yeast alcohol dehydrogenase gene (Figure 1). Approximately 3 x 10<sup>6</sup> transformants were screened for the ability to grow on glucose containing medium. After 12 days of incubation, twelve colonies were obtained. The majority of these proved to be revertants. However, in two cases, the ability to grow on glucose correlated with the maintenance of the LEU2 marker as assessed by plasmid stability tests. These two yeast transformants carried plasmids designated pCYCD1-21 and pCYCD1-19 (see below). Both were recovered in E. coli. Upon reintroduction into yeast, the plasmids rescued the CLN deficient strain, although the rescue was inefficient and the rescued strain grew relatively poorly.

The restriction map and partial DNA sequence analysis revealed that pCYCD1-19 and pCYCD1-21 were independent clones representing the same gene. The 1.2 kb insert of pCYCD1-21 was used as probe to screen a human HeLa cDNA library for a full length cDNA clone. Approximately 2 million cDNA clones were screened and 9 positives were obtained. The longest one of these clones, pCYCD1-H12 (1325 bp), was completely sequenced (Figure 2). The sequence exhibits a very high GC content within the coding region (61%) and contains a poly A tail (69 A residues). The estimated molecular weight of the predicted protein product of the gene is 33,670 daltons starting from the first in-frame AUG codon at nucleotide 145 (Figure 2). The predicted protein is related to other cyclins (see below) and has an unusually low pI of 4.9 (compared to 6.4 of human cyclin A, 7.7 of human cyclin B and 5.6 of CLN1), largely contributed by the high concentration of acidic residues at its C-terminus.

There are neither methionine nor stop codons 5' to the predicted initiating methionine at nucleotide 145. Because of this and also because of the apparent N-terminal

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truncation of CYCD1 with respect to other cyclins (see below for more detail), four additional human cDNA libraries were further screened to see if the  $\lambda$ CYCD1-H12 clone might lack the full 5' region of the cDNA. Among more than 100 cDNA clones isolated from these screens, none was found that had a more extensive 5' region than that of  $\lambda$ CYCD1-H12. The full length coding capacity of clone H12 was later confirmed by Western blot analysis (see below).

CYCD1 encodes the smallest (34 kd) cyclin protein identified so far, compared to the 49 kd human cyclin A, 50 kd human cyclin B and 62 kd *S. cerevisiae* CLN1. By comparison with A and B type cyclins, the difference is due to the lack of almost the entire N-terminal segment that contains the so called "destruction box" identified in both A and B type cyclins (Glotzer M. et al., Nature 349:132 (1991)).

#### Sequence Analysis of D1 and Comparison with Other Cyclins

Sequence analysis revealed homology between the CYCD1-H12 encoded protein and other cyclins. However, it is clear that CYCD1 differs from the three existing classes of cyclins, A, B and CLN. To examine how this new cyclin gene might be evolutionary related to other cyclins, a comprehensive amino acid sequence comparison of all cyclin genes was conducted. Fifteen previously published cyclin sequences as well as CYCD1 were first aligned using a strategy described in detail by Xiong and Eickbush (Xiong, Y. and et al., EMBO J. 9:3353 (1990)). Effort was made to reach the maximum similarity between sequences with the minimum introduction of insertion/deletions and to include as much sequence as possible. With the exception of CLN cyclins, this alignment contains about 200 amino acids residues which occupies more than 70% of total coding region of CYCD1 (Figure 5A). There is a conserved domain and some scattered similarities between members of A and B type cyclins N-terminal to the aligned region (Glotzer, M. et al., Nature 349:132 (1991)), but this is not present in

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either CLN cyclins or CYCD1 and CYL1 and so they were not included in the alignment.

The percent divergence for all pairwise comparisons of the 17 aligned sequences was calculated and used to construct an evolutionary tree of cyclin gene family using the Neighbor-Joining method (Saitou, N., et al., Mol. Biol. Evol. 4:406 (1987) and Experimental Procedures). Because of the lowest similarity of CLN cyclins to the other three classes, the tree (Figure 5B) was rooted at the connection between the CLN cyclins and the others. It is very clear from this evolutionary tree that CYCD1, CYCD2 and CYCD3 represent a distinct new class of cyclin, designated cyclin D.

EXAMPLE 2:      Expression of the Cyclin D1  
                    Gene in Human Cells

Expression of cyclin D1 gene in human cells was studied by Northern analysis. Initial studies indicated that the level of cyclin D1 expression was very low in several cell lines. Poly (A)<sup>+</sup>RNA was prepared from HeLa cells and probed with the entire coding region of CYCD1 gene. Two major transcripts of 4.8 kb and 1.7 kb were detected. The high molecular weight form was the most abundant. With the exception of a few cDNA clones, which were truncated at either the 5' or 3' ends, most of the cDNA clones isolated from various different cDNA libraries are very similar to the clone  $\lambda$ CYCD1-H12 (Figure 2). Thus, it appears that the 1.7 kb transcript detected in Northern blots corresponds to nucleotide sequence in Figure 2.

To understand the origin of the larger 4.8 kb transcript, both 5' and 3' end sub-fragments of the  $\lambda$ CYCD1-H12 clone were used to screen both cDNA and genomic libraries, to test whether there might be alternative transcription initiation, polyadenylation and/or mRNA splicing. Two longer cDNA clones,  $\lambda$ CYCD1-HO34 (1.7 kb) from HeLa cells and  $\lambda$ DYDC1-TO78 (4.1 kb) from human teratocarcinoma cells, as well as several genomic clones were isolated and partially

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sequenced. Both  $\lambda$ CYCD1-HO34 and  $\lambda$ CYCD1-TO78 have identical sequences to  $\lambda$ CYCD1-H12 clone from their 5' ends (Figure 6). Both differ from  $\lambda$ CYCD1-H12 in having additional sequences at the 3' end, after the site of polyadenylation. These 3' sequences are the same in  $\lambda$ CYCD1-HO34 and  $\lambda$ CYCD1-TO78, but extend further in the latter clone (Figure 6). Nucleotide sequencing of a genomic clone within this region revealed colinearity between the cDNAs and the genomic DNA (Figure 6). There is a single base deletion (an A residue) in  $\lambda$ CYCD1-TO78 cDNA clone. This may be the result of polymorphism, although it is not possible to exclude the possibility that some other mechanism is involved. The same 4.8 kb transcript, but not the 1.7 kb transcript, was detected using the 3' end extra fragment from clone TO78 as a probe.

It appears that the two mRNAs detected in Northern blots arise by differential polyadenylation (Figure 6). Strangely, there is no recognizable polyadenylation sequence (AAUAAA) anywhere within the sequence of clone  $\lambda$ CYCD1-H12, even though polyadenylation has clearly occurred (Figure 2). There is also no close variant of AAUAAA (nothing with less than two mismatches).

EXAMPLE 3:      Differential Expression of Cyclin D1 Gene in Different Cell Types

During the screening of cDNA libraries to obtain full length clones of CYCD1, it became evident that the cDNA library derived from the human glioblastoma cell line (U118 MG) from which the yeast transformants were obtained gave rise to many more positives than the other four cDNA libraries. Northern and Western blotting were carried out to explore the possibility that cyclin D1 might be differentially expressed in different tissues or cell lines. Total RNA was isolated from U118 MG cells and analyzed by Northern blot using the CYCD1 gene coding region as probe. The level of transcript is 7 to 10 fold higher in the glioblastoma cells,



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compared to HeLa cells. In both HeLa and U118 MG cells, both high and low molecular weight transcripts are observed.

To investigate whether the abundant CYCD1 message in the U118 MC cell line is reflected at the protein level, cell  
5 extracts were prepared and Western blotting was performed using anti-CYL1 prepared against mouse CYL1 (provided by Matsushime, H. et al.). This anti-CYL1 antibody was able to detect nanogram quantities of recombinant CYCD1 on Western blots (data not shown), and was also able to detect CYCD1 in  
10 the original yeast transformants by immunoprecipitation and Western analysis. Initial experiments using total cell extracts, from HeLa, 293 or U118 MG cells failed to detect any signal. However, if the cell extracts were immunoprecipitated with the serum before being subjected to  
15 SDS-PAGE and immunoblotting, a 34 kd polypeptide was readily detected in U118 NC cells. The protein is far less abundant in HeLa cells and was not detectable in 293 cells. The molecular weight of the anti-CYCL1 cross-reactive material from U118 MG and HeLa is exactly that of the human CYCD1  
20 protein expressed in E. coli. This argues that the sequenced cDNA clones contain the entire open reading frame.

#### EXPERIMENTAL PROCEDURES

##### Strain Construction

The parental strain was BF305-15d (MATa leu2-3 leu2-112  
25 his3-11 his3-15 ura3-52 trp1 ade1 met14 arg5,6) (Futcher, B., et al., Mol. Cell. Biol. 6:2213 (1986)). The strain was converted into a conditional cln- strain in three steps. First, the chromosomal CLN3 gene was placed under control of the GAL1 promoter. A 0.75 kb EcoRI-BamHI fragment  
30 containing the bidirectional GAL10-GAL1 promoters was fused to the 5' end of the CLN3 gene, such that the BamHI (GAL1) end was attached 110 nucleotides upstream of the CLN3 start codon. An EcoRI fragment stretching from the GAL10 promoter to the middle of CLN3 (Nash, R. et al., EMBO J. 7:4335

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(1988)) was then subcloned between the XhoI and EcoRI sites of pBF30 (Nash, R. et al., EMBO J 7:4335 (1988)). The ligation of the XhoI end to the EcoRI end was accomplished by filling in the ends with Klenow, and blunt-end ligating  
5 (destroying the EcoRI site). As a result, the GAL1 promoter had replaced the DNA normally found between -110 and -411 upstream of CLN3. Next, an EcoRI to SphI fragment was excised from this new pBF30 derivative. This fragment had extensive 5' and 3' homology to the CLN3 region, but  
10 contained the GAL1 promoter and a URA3 marker just upstream of CLN3. Strain BF305-15d was transformed with this fragment and Ura<sup>+</sup> transformants were selected. These were checked by Southern analysis. In addition, average cell size was measured when the GAL1 promoter was induced or  
15 uninduced. When the GAL1 promoter was induced by growing the cells in 1% raffinose and 1% galactose, mode cell volume was about 25 $\mu$ m<sup>3</sup> (compared to a mode volume of about 40 $\mu$ m<sup>3</sup> for the parental strain) whereas when the promoter was not induced (raffinose alone), or was repressed by the presence  
20 of glucose, cell volume was much larger than for the wildtype strain. These experiments showed that CLN3 had been placed under control of the GAL1 promoter. It is important to note that this GAL1-controlled, glucose repressible gene is the only source of CLN3 protein in the  
25 cell.

Second, the CLN1 gene was disrupted. A fragment of CLN1 was obtained from I. Fitch, and used to obtain a full length clone of CLN1 by hybridization, and this was subcloned into a pUC plasmid. A BamHI fragment carrying the HIS3 gene was  
30 inserted into an NcoI site in the CLN1 open reading frame. A large EcoRI fragment with extensive 5' and 3' homology to the CLN1 region was then excised, and used to transform the BF305-15d GAL-CLN3 strain described above. Transformation was done on YNB-his raffinose galactose plates. His<sup>+</sup> clones  
35 were selected, and checked by Southern analysis.

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Finally, the CLN2 gene was disrupted. A fragment of CLN2 was obtained from I. Fitch, and used to obtain a full length clone of CLN2 by hybridization, and this was subcloned into a pUC plasmid. An EcoRI fragment carrying the TRP1 gene was inserted into an SpeI site in the CLN2 open reading frame. A BamHI-KpnI fragment was excised and used to transform the BF305-15d GAL-CLN3 HIS3::cln1 strain described above. Transformation was done on YNB-trp raffinose galactose plates. Trp+ clones were selected. In this case, because the TRP1 fragment included an ARS, many of the transformants contained autonomously replicating plasmid rather than a disrupted CLN2 gene. However, several percent of the transformants were simple TRP1::cln2 disruptants, as shown by phenotypic and Southern analysis.

One particular 305-15d GAL1-CLN3 HIS3::cln1 TRP1::cln2 transformant called clone #21 (referred to hereafter as 305-15d #21) was analyzed extensively. When grown in 1% raffinose and 1% galactose, it had a doubling time indistinguishable from the CLN wild-type parental strain. However, it displayed a moderate Wee phenotype (small cell volume), as expected for a CLN3 overexpressor. When glucose was added, or when galactose was removed, cells accumulated in G1 phase, and cell division ceased, though cells continued to increase in mass and volume. After overnight incubation in the G1-arrested state, essentially no budded cells were seen, and a large proportion of the cells had lysed due to their uncontrolled increase in size.

When 305-15d #21 was spread on glucose plates, revertant colonies arose at a frequency of about  $10^{-7}$ . The nature of these glucose-resistant, galactose-independent mutants was not investigated.

#### Yeast Spheroplasts Transformation

S. cerevisiae spheroplasts transformation was carried out according to Burgers and Percival and Allshire (Burgers,

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P.M.J. et al., Anal. Biochem. 163:391 (1987); Allshire, R.C., Proc. Natl. Acad. Sci. USA 87:4043 (1990)).

### Cell Culture

HeLa and 293 cells were cultured at 37°C either on plates or  
5 in suspension in Dulbecco's modified Eagle's medium (DMEM)  
supplemented with 10% fetal calf serum. Glioblastoma U118  
MG cells were cultured on plates in DMEM supplemented with  
15% fetal bovine serum and 0.1 mM non-essential amino acid  
(GIBCO).

### 10 Nucleic Acid Procedures

Most molecular biology techniques were essentially the same  
as described by Sambrook, et al. (Sambrook, J. et al.,  
Molecular Cloning: A Laboratory Manual Cold Spring Harbor  
Laboratory, Cold Spring Harbor, NY (1989)). Phagmid vectors  
15 pUC118 or pUC119 (Vieira, J. et al., Meth. Enzymol. 153:3  
(1987)) or pBlueScript (Stratagene) were used as cloning  
vectors. DNA sequences were determined either by a chain  
termination method (Sanger, F. et al., Proc. Natl. Acad.  
Sci. USA 74:5463 (1977)) using Sequenase Kit (United States  
20 Biochemical) or on an Automated Sequencing System (373A,  
Applied Biosystems).

Human HeLa cell cDNA library in  $\lambda$ ZAP II was purchased from  
Stratagene. Human T cell cDNA library in  $\lambda$ gt10 was a gift  
of M. Gillman (Cold Spring Harbor Laboratory). Human  
25 glioblastoma U118 MG and glioblastoma SW1088 cell cDNA  
libraries in  $\lambda$ ZAP II were gifts of M. Wigler (Cold Spring  
Harbor Laboratory). Human teratocarcinoma cell cDNA library  
 $\lambda$ gt10 was a gift of Skowronski (Cold Spring Harbor  
Laboratory). Normal human liver genomic library  $\lambda$ GEM-11 was  
30 purchased from Promega.

Total RNA from cell culture was extracted exactly according  
to Sambrook, et al. (Sambrook, J. et al., Molecular

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Cloning: A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)) using guanidium thiocyanate followed by centrifugation in CsCl solution. Poly(A)+RNA was isolated from total RNA preparation using Poly (A)+Quick push columns (Stratagene). RNA samples were separated on a 1% agarose-formaldehyde MOPs gel and transferred to a nitrocellulose filter. Northern hybridizations (as well as library screening) were carried out at 68°C in a solution containing 5 x Denhardt's solution, 2 x SSC, 0.1% SDS, 100 10 µg/ml denatured Salmon sperm DNA, 25 µM NaPO<sub>4</sub> (pH7.0) and 10% dextran sulfate. Probes were labelled by the random priming labelling method (Feinberg, A. et al., Anal. Biochem. 132:6 (1983)). A 1.3 kb Hind III fragment of cDNA clone pCYCD1H12 was used as coding region probe for Northern 15 hybridization and genomic library screening, a 1.7 kb Hind III-EcoRI fragment from cDNA clone pCYCD1-TO78 was used as 3' fragment probe.

To express human cyclin D1 gene in bacteria, a 1.3 kb Nco I-Hind II fragment of pCYCD1-H12 containing the entire CYCD1 20 open reading frame was subcloned into a T7 expression vector (pET3d, Studier, F.W. et al., Methods in Enzymology 185:60 (1990)). Induction of E. coli strain BL21 (DE3) harboring the expression construct was according to Studier (Studier, F.W. et al., Methods in Enzymology 185:60 (1990)). Bacterial 25 culture was lysed by sonication in a lysis buffer (5 mM EDTA, 10% glycerol, 50 mM Tris-HCL, pH 8.0, 0.005% Triton X-100) containing 6 M urea (CYCD1 encoded p34 is only partial soluble in 8 M urea), centrifuged for 15 minutes at 20,000 g force. The pellet was washed once in the lysis buffer 30 with 6 M urea, pelleted again, resuspended in lysis buffer containing 8 urea, and centrifuged. The supernatant which enriched the 34 kd CYCD1 protein was loaded on a 10% polyacrymide gel. The 34 kd band was cut from the gel and eluted with PBS containing 0.1% SDS.

Sequence Alignment and Formation of an Evolutionary Tree

Protein sequence alignment was conducted virtually by eye according to the methods described and discussed in detail by Xiong and Eickbush (Xiong, Y. et al., EMBO J. 9:3353 (1990)). Numbers within certain sequences indicate the number of amino acid residues omitted from the sequence as the result of insertion.

Numbers within certain sequences indicate the number of amino acid residues omitted from the sequence as the result of insertion (e.g., for CLN1, ...TWG25RLS...- indicates that 25 amino acids have been omitted between G and R). Sources for each sequence used in this alignment and in the construction of an evolutionary tree (Figure 5B) are as follows: CYCA-Hs, human A type cyclin (Wang, J. et al., Nature 343:555 (1990)); CYCA-X1, *Xenopus* A-type cyclin (Minshull, J. et al., EMBO J. 9:2865 (1990)); CYCA-Ss, clam A-type cyclin (Swenson, K.I. et al., Cell 47:867 (1986); CYCA-Dm, *Drosophila* A-type cyclin (Lehner, C.F. et al., Cell 56:957 (1989)); CYCB1-Hs, human B1-type cyclin (Pines, J. et al., Cell 58:833 (1989); CYCB1-X1 and CYCB2-X1, *Xenopus* B1- and B2-type cyclin (Minshull, J. et al., Cell 56:947-956 (1989)); CYCB-Ss, clam B-type cyclin (Westendorf, J.M et al., J Cell Biol. 108:1431 (1989)); CYCB-Asp, starfish B-type cyclin (Tachibana, K. et al., Dev. Biol. 140:241 (1990)); CYCB-Arp, sea urchin B-type cyclin (Pines, J. et al., EMBO J. 6:2987 (1987)); CYCB-Dm, *Drosophila* B-type cyclin (Lehner, C.F. et al., Cell 61:535 (1990)); CDC13-Sp, *S. pombe* CDC13 (Booher, R. et al., EMBO J. 7:2321 (1988)); CLN1-Sc and CLN2-Sc, *S. cerevisiae* cyclin 1 and 2 (Hadwiger, J.A. et al., Proc. Natl. Acad. Sci. USA 86:6255 (1989)); CLN3-Sc, *S. cerevisiae* cyclin 3 (Nash, R. et al., EMBO J. 7:4335 (1988)).

A total of 17 cyclin sequences were aligned and two representative sequences from each class are presented in Figure 5A.

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Percent divergence of all pairwise comparison of 17 sequences were calculated from 154 amino acid residues common to all 17 sequences, which does not include the 50 residue segments located at N-terminal part of A, B and D-type cyclins because of its absence from CLN type cyclins. A gap/insertion was counted as one mismatch regardless of its size. Before tree construction, all values were changed to distance with Poisson correction ( $d = -\log_{es}$ , where the  $S$  = sequence similarity (Nei, M. Molecular Evolutionary Genetics pp. 287-326 Columbia University Press, NY (1987)). Calculation of pairwise comparison and Poisson correction were conducted using computer programs developed at University of Rochester. Evolutionary trees of cyclin gene family was generated by the Neighbor-Joining program (Saitou, N. et al., Mol. Biol. Evol. 4:406 (1987)). All calculations were conducted on VAX computer MicroVMS V4.4 of Cold Spring Harbor Laboratory. The reliability of the tree was evaluated by using a subset sequence (e.g., A, B and D-type cyclins), including more residues (e.g., the 50-residue segment located at C-terminal of A, B and D-type cyclins, Figure 5A) or adding several other unpublished cyclin sequences. They all gave rise to the tree with the same topology as the one presented in Figure 5B.

#### Immunoprecipitation and Western Blots

Cells from 60 to 80% confluent 100 mm dish were lysed in 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20 mM EDTA, 0.5% NP-40, 0.5% Nadeoxycholate, 1 mM PMSF) for 30 minutes on ice. Immunoprecipitation was carried out using 1 mg protein from each cell lysate at 4°C for overnight. After equilibrated with the lysis buffer, 60  $\mu$ l of Protein A-agarose (PIERCE) was added to each immunoprecipitation and incubated at 4°C for 1 hour with constant rotating. The immunoprecipitate was washed three times with the lysis buffer and final resuspended in 50  $\mu$ l 2 x SDS protein sample buffer boiled for 5 minutes and loaded onto a 10% polyacrymide gel. Proteins were transferred to a

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nitrocellulose filter using a SDE Electroblothing System (Millipore) for 45 minutes at a constant current of 400 mA. The filter was blocked for 2 to 6 hours with 1 x PBS, 3% BSA and 0.1% sodium azide, washed 10 minutes each time and 6 times with NET gel buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% NP-40, 1 mM EDTA, 0.25% gelatin and 0.02 sodium azide), radio-labelled with <sup>125</sup>I-Protein A for 1 hour in blocking solution with shaking. The blot was then washed 10 minutes each time and 6 times with the NET gel buffer before  
10 autoradiography.

The tree was constructed using the Neighbor-Joining method (Saitou, N. et al., Mol. Biol. Evol. 4:406 (1987)). The length of horizontal line reflects the divergence. The branch length between the node connecting the CLN cyclins  
15 and other cyclins was arbitrarily divided.

#### MATERIALS AND METHODS

The following materials and methods were used in the work described in Examples 4-6.

#### Molecular Cloning

20 The human HeLa cell cDNA library, the human glioblastoma cell U118 MG cDNA library, the normal human liver genomic library, and the hybridization buffer were the same as those described above. A human hippocampus cDNA library was purchased from Stratagene, Inc. High and low-stringency  
25 hybridizations were carried out at 68° and 50°C, respectively. To prepare template DNA for PCR reactions, approximately 2 million lambda phages from each cDNA library were plated at a density of 10<sup>5</sup> PFU/150-mm plate, and DNA was prepared from the plate lysate according to Sambrook, J.  
30 et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.



EXAMPLE 4: Isolation of Human Cyclin D2 and D3 cDNAs

To isolate human cyclin D2 and D3 cDNAs, two 5' oligonucleotides and one 3' degenerate oligonucleotide were derived from three highly conserved regions of human CCND1, mouse cyl1, cyl2, and cyl3 D-type cyclins (Matsushime, H. et al., Cell 65:701 (1991); Xiong, Y. et al., Cell 65:691; Figure 8). The first 5' oligonucleotide primer, HCND11, is a 8192-fold degenerate 38-mer (TGGATG [T/C] TNGA [A/G] GTNTG [T/C] GA [A/C] GA [A/G] CA- [A/G] AA [A/G] TG [T/C] GA [A/G] GA) (SEQ ID No. 37), encoding 13 amino acids (WMLEVCEEQKCEE) (SEQ ID No. 38). The second 5' oligonucleotide primer, HCND12, is a 8192-fold degenerate 29-mer (GTNTT [T/C] CCN [T/C] TNGCNATGAA [T/C] TA [T/C] TNGA) (SEQ ID No. 39), encoding 10 amino acids (VFPLAMNYLD) (SEQ ID No. 40). The 3' primer, HCND13, is a 3072-fold degenerate 24-mer ([A/G] TCNGT [A/G] TA [A/G/T] AT [A/G] CANA [A/G] [T/C] TT- [T/C] TC) (SEQ ID No. 41), encoding 8 amino acids (EKLCIYTD) (SEQ ID No. 42). The PCR reactions were carried out for 30 cycles at 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min. The reactions contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 0.01% gelatin, 0.2 mM each of dATP, dGTP, dCTP, and dTTP, 2.5 units of Taq polymerase, 5 μM of oligonucleotide, and 2-10 μg of template DNA. PCR products generated by HCND11 and HCND13 were verified in a second-round PCR reaction using HCND12 and HCND13 as the primers. After resolution on a 1.2% agarose gel, DNA fragments with the expected size (200 bp between primer HCND11 and HCND13) were purified and subcloned into the SmaI site of phagmid vector pUC118 for sequencing.

To isolate full-length cyclin D3 cDNA, the 201-bp fragment of the D3 PCR product was labeled with oligonucleotide primers HCND11 and HCND13 using a random-primed labeling technique (Feinberg, A. P. et al., Anal. Biochem. 132:6 (1983)) and used to screen a human HeLa cell cDNA library. The probe used to screen the human genomic library for the CCND3 gene was a 2-kb EcoRI fragment derived from cDNA

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clone  $\lambda$ D3-H34. All hybridizations for the screen of human cyclin D3 were carried out at high stringency.

The PCR clones corresponding to CCND1 and CCND3 have been repeatedly isolated from both cDNA libraries; CCND2 has not.

- 5 To isolate cyclin D2, a 1-kb EcoRI fragment derived from mouse cy12 cDNA was used as a probe to screen a human genomic library. Under low-stringency conditions, this probe hybridized to both human cyclins D1 and D2. The cyclin D1 clones were eliminated through another
- 10 hybridization with a human cyclin D1 probe at high stringency. Human CCND2 genomic clones were subsequently identified by partial sequencing and by comparing the predicted protein sequence with that of human cyclins D1 and D3 as well as mouse cy12.

- 15 As described above, human CCND1 (cyclin D1) was isolated by rescuing a triple Cln deficiency mutant of Saccharomyces cerevisiae using a genetic complementation screen. Evolutionary proximity between human and mouse, and the high sequence similarity among cy11, cy12, and cy13, suggested
- 20 the existence of two additional D-type cyclin genes in the human genome. The PCR technique was first used to isolate the putative human cyclin D2 and D3 genes. Three degenerate oligonucleotide primers were derived from highly conserved regions of human CCND1, mouse cy11, cy12, and cy13. Using
- 25 these primers, cyclin D1 and a 200-bp DNA fragment that appeared to be the human homolog of mouse cy13 from both human HeLa cell and glioblastoma cell cDNA libraries was isolated. A human HeLa cell cDNA library was screened with this PCR product as probe to obtain a full-length D3 clone.
- 30 Some 1.2 million cDNA clones were screened, and six positives were obtained. The longest cDNA clone from this screen,  $\lambda$ D3-H34 (1962 bp), was completely sequenced (Figure 4).

- Because a putative human cyclin D2 cDNA was not detected by
- 35 PCR, mouse cy12 cDNA was used as a heterologous probe to

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screen a human cDNA library at low stringency. This resulted, initially, in isolation of 10 clones from the HeLa cell cDNA library, but all corresponded to the human cyclin D1 gene on the basis of restriction mapping. Presumably, this was because cyclin D2 in HeLa cells is expressed at very low levels. Thus, the same probe was used to screen a human genomic library, based on the assumption that the representation of D1 and D2 should be approximately equal. Of the 18 positives obtained, 10 corresponded to human cyclin D1 and 8 appeared to contain human cyclin D2 sequences (see below). A 0.4-kb BamHI restriction fragment derived from  $\lambda$ D2-G1 1 of the 8 putative cyclin D2 clones, was then used as probe to screen a human hippocampus cDNA library at high stringency to search for a full-length cDNA clone of the cyclin D2 gene. Nine positives were obtained after screening of approximately 1 million cDNA clones. The longest cDNA clone,  $\lambda$ D2-P3 (1911 bp), was completely sequenced (Figure 3). Neither  $\lambda$ D2-P3 nor  $\lambda$ D3-H34 contains a poly(A) sequence, suggesting that part of the 3' untranslated region might be missing.

The DNA sequence of  $\lambda$ D2-P3 revealed an open reading frame that could encode a 289-amino-acid protein with a 33,045-Da calculated molecular weight. A similar analysis of  $\lambda$ D3-H34 revealed a 292-amino-acid open reading frame encoding a protein with a 32,482-Da calculated molecular weight. As in the case of human cyclin D1, there is neither methionine nor stop codons 5' to the presumptive initiating methionine codon for both  $\lambda$ D2-P3 (nucleotide position 22, Figure 3) and  $\lambda$ D3-H34 (nucleotide position 101, Figure 4). On the basis of the protein sequence comparison with human cyclin D1 and mouse cy11 (Figure 7) and preliminary results of the RNase protection experiment, both  $\lambda$ D2-P3 and  $\lambda$ D3-H34 are believed to contain full-length coding regions.

The protein sequence of all 11 mammalian cyclins identified to date were compared to assess their structural and evolutionary relationships. This includes cyclin A, cyclins

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B1 and B2, six D-type cyclins (three from human and three from mouse), and the recently identified cyclins E and C (Figure 7). Several features concerning D-type cyclins can be seen from this comparison. First, as noted previously for cyclin D1, all three cyclin D genes encode a similar small size protein ranging from 289 to 295 amino acid residues, the shortest cyclins found so far. Second, they all lack the so-called "destruction box" identified in the N-terminus of both A- and B-type cyclins, which targets it for ubiquitin-dependent degradation (Glutzer, M. et al., Nature 349:132 (1991)). This suggests either that the D-type cyclins have evolved a different mechanism to govern their periodic degradation during each cell cycle or that they do not undergo such destruction. Third, the three human cyclin D genes share very high similarity over their entire coding region: 60% between D1 and D2, 60% between D2 and D3, and 52% between D1 and D3. Fourth, members of the D-type cyclins are more closely related to each other than are members of the B-type cyclins, averaging 78% for three cyclin D genes in the cyclin box versus 57% for two cyclin B genes. This suggests that the separation (emergence) of D-type cyclins occurred after that of cyclin B1 from B2. Finally, using the well-characterized mitotic B-type cyclin as an index, the most closely related genes are cyclin A (average 51%), followed by the E-type (40%), D-type (29%), and C-type cyclins (20%).

EXAMPLE 5: Chromosome Localization of CCND2 and CCND3

The chromosome localization of CCND2 and CCND3 was determined by fluorescence in situ hybridization. Chromosome in situ suppression hybridization and in situ hybridization banding were performed as described previously (Lichter, T. et al., Science 247:64 (1990); Baldini, A. et al., Genomics 9:770 (1991)). Briefly  $\lambda$ D2-G4 and  $\lambda$ D3-G9 lambda genomic DNAs containing inserts of 15 and 16 kb, respectively, were labeled with biotin-11-dUTP (Sigma) by nick-translation (Brigatti, D. J. et al., Urology 126:32 (1983); Boyle, A.

L., In Current Protocols in Molecular Biology, Wiley, New York, 1991). Probe size ranged between 200 and 400 nucleotides, and unincorporated nucleotides were separated from probes using Sephadex G-50 spin columns (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). Metaphase chromosome spreads prepared by the standard technique (Lichter, T. et al., Science 247:64 (1990)) were hybridized in situ with biotin-labeled D2-G4 or D3-G9. Denaturation and preannealing of 5  $\mu$ g of DNase-treated human placental DNA, 7  $\mu$ g of DNased salmon sperm DNA, and 100 ng of labeled probe were performed before the cocktail was applied to Alu prehybridized slides. The in situ hybridization banding pattern used for chromosome identification and visual localization of the probe was generated by cohybridizing the spreads with 40 ng of an Alu 48-mer oligonucleotide. This Alu oligo was chemically labeled with digoxigenin-11-dUTP (Boehringer-Mannheim) and denatured before being applied to denatured chromosomes. Following 16-18 h of incubation at 37°C and posthybridization wash, slides were incubated with blocking solution and detection reagent (Lichter, T. et al., Science 247:64 (1990)). Biotin-labeled DNA was detected using fluorescence isothiocyanate (FITC)-conjugated avidin DCS (5  $\mu$ g/ml) (Vector Laboratories); digoxigenin-labeled DNA was detected using a rhodamine-conjugated anti-digoxigenin antibody (Boehringer-Mannheim). Fluorescence signals were imaged separately using a Zeiss Axioskop-20 epifluorescence microscope equipped with a cooled CCD camera (Photometrics CH220). Camera control and image acquisition were performed using an Apple Macintosh IIX computer. The gray scale images were pseudocolored and merged electronically as described previously (Baldini, A. et al., Genomics 9:770 (1991)). Image processing was done on a Macintosh IICI computer using Gene Join Maxpix (software by Tim Rand in the laboratory of D. Ward, Yale) to merge FITC and rhodamine images. Photographs were taken directly from the computer monitor.

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Chromosomal fluorescence in situ hybridization was used to localize D2-G4 and D3-G9. The cytogenetic location of D2-G4 on chromosome 12p band 13 and that of D3-G9 on chromosome 6p band 21 were determined by direct visualization of the two-color fluorescence in situ hybridization using the biotin-labeled probe and the digoxigen-labeled Alu 48-mer oligonucleotide (Figure 5).

The Alu 48-mer R-bands, consistent with the conventional R-banding pattern, were imaged and merged with images generated from the D2-G4 and D3-G9 hybridized probes. The loci of D2-G4 and D3-G9 were visualized against the Alu banding by merging the corresponding FITC and rhodamine images. This merged image allows the direct visualization of D2-G4 and D3-G9 on chromosomes 12 and 6, respectively. The D2-G4 probe lies on the positive R-band 12p13, while D3-G9 lies on the positive R-band 6p21.

Cross-hybridization was not detected with either pseudogene cyclin D2 or D3, presumably because the potentially cross-hybridizing sequence represents only a sufficiently small proportion of the 15- and 16-kb genomic fragments (nonsuppressed) used as probe, and the nucleotide sequences of pseudo genes have diverged from their ancestral active genes.

**EXAMPLE 6:** Isolation and Characterization of  
Genomic Clones of Human D-Type Cyclins

Genomic clones of human D-type cyclins were isolated and characterized to study the genomic structure and to obtain probes for chromosomal mapping. The entire 1.3-kb cyclin D1 cDNA clone was used as probe to screen a normal human liver genomic library. Five million lambda clones were screened, and three positives were obtained. After initial restriction mapping and hybridizations, lambda clone G6 was chosen for further analysis. A 1.7-kb BamHI restriction fragment of  $\lambda$ D1-G6 was subcloned into pUC118 and completely sequenced. Comparison with the cDNA clones previously

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isolated and RNase protection experiment results (Withers, D.A. et al., Mol. Cell. Biol. 11:4846 (1991)) indicated that this fragment corresponds to the 5' part of the cyclin D1 gene. As shown in Figure 8A, it contains 1150 bp of upstream promoter sequence and a 198-bp exon followed by an intron.

Eighteen lambda genomic clones were isolated from a similar screening using mouse cy12 cDNA as a probe under low-stringency hybridization conditions, as described above (Example 4). Because it was noted in previous cDNA library screening that the mouse cy12 cDNA probe can cross-hybridize with the human D1 gene at low stringency, a dot-blot hybridization at high stringency was carried out, using the human D1 cDNA probe. Ten of the 18 clones hybridized with the human D1 probe and 8 did not. On the basis of the restriction digestion analysis, the 8 lambda clones that did not hybridize with the human D1 probe at high stringency fall into three classes represented by  $\lambda$ D2-G1,  $\lambda$ D2-G2, and  $\lambda$ D2-G4, respectively. These three lambda clones were subcloned into a pUC plasmid vector, and small restriction fragments containing coding region were identified by Southern hybridization using a mouse cy12 cDNA probe. A 0.4-kb BamHI fragment derived from  $\lambda$ D2-G1 was subsequently used as a probe to screen a human hippocampus cell cDNA library at high stringency. Detailed restriction mapping and partial sequencing indicated that  $\lambda$ D2-G1 and  $\lambda$ D2-G2 were two different clones corresponding to the same gene, whereas  $\lambda$ D2-G4 appeared to correspond to a different gene. A 2.7-kb SacI-SmaI fragment from  $\lambda$ D2-G4 and 1.5-kb BclI-BglII fragment from  $\lambda$ D2-G1 have been completely sequenced. Nucleotide sequence comparison revealed that the clone  $\lambda$ D2-G4 corresponds to the D2 cDNA clone  $\lambda$ D2-P3 (Figure 3). As shown in Figure 8A, the 2.7-kb SacI-SmaI fragment contains 1620 bp of sequence 5' to the presumptive initiating methionine codon identified in D2 cDNA (Figure 3) and a 195-bp exon followed by a 907-bp intervening sequence.

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Lambda genomic clones corresponding to the human cyclin D3 were isolated from the same genomic library using human D3 cDNA as a probe. Of four million clones screened, nine were positives. Two classes of clones, represented by  $\lambda$ D3-G4 and  $\lambda$ D3-G9, were distinguished by restriction digestion analysis. A 2.0-kb HindIII-ScaI restriction fragment from  $\lambda$ D3-G5 and a 3.7-kb SacI-HindIII restriction fragment from  $\lambda$ D3-G9 were further subcloned into a pUC plasmid vector for more detailed restriction mapping and complete sequencing, as they both hybridized to the 5' cyclin D3 cDNA probe. As presented in Figure 9C, the 3.7-kb fragment from clone G9 contains 1.8 kb of sequence 5' to the presumptive initiating methionine codon identified in D3 cDNA (Figure 4), a 198-bp exon 1, a 684-bp exon 2, and a 870-bp intron.

Comparison of the genomic clones of cyclins D1, D2, and D3 revealed that the coding regions of all three human CCND genes are interrupted at the same position by an intron (indicated by an arrow in Figure 8). This indicated that the intron occurred before the separation of cyclin D genes.

EXAMPLE 7: Isolation and Characterization of Two Cyclin D Pseudogenes

The 1.5-kb BclI-BglII fragment subcloned from clone  $\lambda$ D2-G1 has been completely sequenced and compared with cyclin D2 cDNA clone  $\lambda$ D2-P3. As shown in Figure 10, it contains three internal stop codons (nucleotide positions 495, 956, and 1310, indicated by asterisks), two frameshifts (position 1188 and 1291, slash lines), one insertion, and one deletion. It has also accumulated many missense nucleotide substitutions, some of which occurred at the positions that are conserved in all cyclins. For example, triplet CGT at position 277 to 279 of D2 cDNA (Figure 3) encodes amino acid Arg, which is an invariant residue in all cyclins (see Figure 8). A nucleotide change from C to T at the corresponding position (nucleotide 731) in clone  $\lambda$ D2-G1 (Figure 10) gave rise to a triplet TGT encoding Cys instead of Arg. Sequencing of the 2.0-kb HindIII-ScaI fragment from



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clone  $\lambda$ D3-G5 revealed a cyclin D3 pseudogene (Figure 11). In addition to a nonsense mutation (nucleotide position 1265), two frameshifts (position 1210 and 1679), a 15-bp internal duplication (underlined region from position 1361 to 1376),  
5 and many missense mutations, a nucleotide change from A to G at position 1182 resulted in an amino acid change from the presumptive initiating methionine codon ATG to GTG encoding Val. On the basis of these analyses, we conclude that clones  $\lambda$ D2-G1 and  $\lambda$ D3-G5 contain pseudogenes of cyclins D2  
10 and D3, respectively.

#### EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention  
15 described herein. Such equivalents are intended to be encompassed by the following claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: MITOTIX
- (ii) TITLE OF INVENTION: D-Type Cyclin and Uses Related Thereto
- (iii) NUMBER OF SEQUENCES: 42
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Hamilton, Brook, Smith & Reynolds, P.C.
  - (B) STREET: Two Militia Drive
  - (C) CITY: Lexington
  - (D) STATE: Massachusetts
  - (E) COUNTRY: US
  - (F) ZIP: 02173
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 07/888,178
  - (B) FILING DATE: 26-MAY-1992
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Granahan, Patricia
  - (B) REGISTRATION NUMBER: 32,227
  - (C) REFERENCE/DOCKET NUMBER: CSHL91-02A
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 617-861-6240
  - (B) TELEFAX: 616-861-9540

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1325 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCAGTAGCAG CGAGCAGCAG AGTCCGCACG CTCCGGCGAG CGCCAGAACA GCGCGAGGGA	60
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AGCTGCCCAG GAAGAGCCCC AGCCATGGAA CACCAGCTCC TGTGCTGCGA AGTGGA AACC	180
ATCCGCCGCG CGTACCCCGA TGCCAACCTC CTCAACGACC GGGTGCTGCG GGCCATGCTG	240
AAGGCGGAGG AGACCTGCGC GCCCTCGGTG TCCTACTTCA AATGTGTGCA GAACGACGTC	300
CTCCCGTCCA TGCCGAAGAT CGTCGCCACC TGGATGCTGG AGGTCTGCGA GGAACAGAAG	360
TGCGAGGAGG AGCTCTTCCC GCTGGCCATG AACTACCTGG ACCGGTTCCT GTCGCTGGAG	420

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CCCGTGAAAA AGAGCCGCCT GCAGCTGCTG GGGGCCACTT GCATGTTTCTT GGCCTCTAAG      480
ATGAAGGAGA CCATCCCCCT GACGGCCGAG AAGCTGTGCA TCTACACCGA CGCCTCCATC      540
CCCCCCGAGG ACCTGCTGCA AATGGAGCTG CTCCTGGTGA ACAAGCTCAA GTGGAACCTG      600
GCCGCAATGA CCCCACACGA TTTCATTGAA CACTTCCTCT CCAAAATGAC AGAGGCGGAG      660
GAGAACAAAC AGATCATCCG CAAACACGCG CAGACCTTCG TTGCCTCTTG TGCCACAGAT      720
CTGAAGTTCA TTTCCAATCC GCCCTCCATG GTGGCAGCGG GGACCGTGGT CGCCGCAGTG      780
CAAGGCCTGA ACCTGAGGAG CCCCAACAAC TTCCTGTCGT ACTACCGCCT CACACGCTTC      840
CTCTCCAGAG TGATCAAGTG TGACCCAGAC TGCCTCCGGG CCTCCCAGGA GCAGATCGAA      900
GCCCTGCTGG AGTCAAGCCT GCGCCAGGCC CACCAGAACA TGGACCCCAA GGCCGCCGAG      960
GAGGAGGAAG AGGAGGAGGA GGAGGTGGAC CTGGCTTGCA CACCCACCGA CGTCCCGGAC     1020
CTGGACATCT GAGGGGCCCA GCGAGGCGGG CGCCACCGCC ACCCGCAGCG AGGGCGGAGC     1080
CGGCCCCAGG TGCTCCACAT GACAGTCCCT CCTCTCCGGA GCATTTTGAT ACCAGAAGGG     1140
AAACCTTCAT TCTCCTTGTT GTTGGTTGTT TTTTCCTTTG CTCTTTCCCC CTTCATCTC     1200
TCACTTAACC AAAACAAAAA GATTACCCAA AAAGTGTCTT TAAAGAGAG AGAGAGAAAA     1260
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA     1320
AAAAA                                             1325

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## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 295 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Glu His Gln Leu Leu Cys Cys Glu Val Glu Thr Ile Arg Arg Ala
 1             5             10             15
Tyr Pro Asp Ala Asn Leu Leu Asn Asp Arg Val Leu Arg Ala Met Leu
          20             25             30
Lys Ala Glu Thr Cys Ala Pro Ser Val Ser Tyr Phe Lys Cys Val
          35             40             45
Gln Lys Glu Val Leu Pro Ser Met Arg Lys Ile Val Ala Thr Trp Met
          50             55             60
Leu Glu Val Cys Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu
65             70             75             80
Ala Met Asn Tyr Leu Asp Arg Phe Leu Ser Leu Glu Pro Val Lys Lys
          85             90             95
Ser Arg Leu Gln Leu Leu Gly Ala Thr Cys Met Phe Val Ala Ser Lys
          100            105            110

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Met Lys Glu Thr Ile Pro Leu Thr Ala Glu Lys Leu Cys Ile Tyr Thr  
115 120 125

Asp Gly Ser Ile Arg Pro Glu Glu Leu Leu Gln Met Glu Leu Leu Leu  
130 135 140

Val Asn Lys Leu Lys Trp Asn Leu Ala Ala Met Thr Pro His Asp Phe  
145 150 155 160

Ile Glu His Phe Leu Ser Lys Met Pro Glu Ala Glu Glu Asn Lys Gln  
165 170 175

Ile Ile Arg Lys His Ala Gln Thr Phe Val Ala Leu Cys Ala Thr Asp  
180 185 190

Val Lys Phe Ile Ser Asn Pro Pro Ser Met Val Ala Ala Gly Ser Val  
195 200 205

Val Ala Ala Val Gln Gly Leu Asn Leu Arg Ser Pro Asn Asn Phe Leu  
210 215 220

Ser Tyr Tyr Arg Leu Thr Arg Phe Leu Ser Arg Val Ile Lys Cys Asp  
225 230 235 240

Pro Asp Cys Leu Arg Ala Cys Gln Glu Gln Ile Glu Ala Leu Leu Glu  
245 250 255

Ser Ser Leu Arg Gln Ala Gln Gln Asn Met Asp Pro Lys Ala Ala Glu  
260 265 270

Glu Glu Glu Glu Glu Glu Glu Glu Val Asp Leu Ala Cys Thr Pro Thr  
275 280 285

Asp Val Arg Asp Val Asp Ile  
290 295

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1970 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCCCGC CGGGCTTGGC CATGGAGCTG CTGTGCCACG AGGTGGACCC GGTCCGCAGG	60
GCCGTGCGGG ACCGCAACCT GCTCGGAGAC GACCGCGTCC TGCAGAACCT GCTCACCATC	120
GAATTCCCGC CGGGCTTGGC CATGGAGCTG CTGTGCCACG AGGTGGACCC GGTCCGCAGG	180
GAGGAGCGCT ACCTTCCGCA GTGCTCCTAC TTCAAGTGCG TGCAGAAGGA CATCCAACCC	240
TACATGCGCA GAATGGTGGC CACCTGGATG CTGGAGGTCT GTGAGGAACA GAAGTGCAGAA	300
GAAGAGGTCT TCCCTCTGGC CATGAATTAC CTGGACCGTT TCTTGGCTGG GGTCCCAGCT	360
CCGAAGTCCC ATCTGCAACT CCTGGGTGCT GTCTGCATGT TCCTGGCCTC CAAACTCAAA	420
GAGACCAGCC CCCTGACCGC GGAGAAGCTG TGCATTTACA CCGACAACCT CATCAAGCCT	480
CAGGAGCTGC TGGAGTGGGA ACTGGTGGTG CTGGGGAAGT TGAAGTGGAA CCTGGCAGCT	540

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GTCACCTCCTC ATGACTTCAT TGAGCACATC TTGCGCAAGC TGCCCCAGCA GCGGGAGAAG      600
CTGTCTCTGA TCCGCAAGCA TGCTCAGACC TTCATTGCTC TGTGTGCCAC CGACTTTAAG      660
TTTGCCATGT ACCCACCCTC GATGATCGCA ACTGGAAGTG TGGGAGCAGC CATCTGTGGG      720
CTCCAGCAGG ATGAGGAAGT GAGCTCGCTC ACTTGTGATG CCCTGACTGA GCTGCTGGCT      780
AAGATCACCA ACACAGACGT GGATTGTCTC AAAGCTTGCC AGGACCAGAT TGAGGCGGTG      840
CTCCTCAATA GCCTGCAGCA GTACCGTCAG GACCAACGTG ACGGATCCAA GTCGGAGGAT      900
GAACTGGACC AAGCCAGCAC CCCTACAGAC GTGCGGGATA TCGACCTGTG AGGATGCCAG      960
TTGGGCCGAA AGAGAGAGAC GCGTCCATAA TCTGGTCTCT TCTTCTTTCT GGTGTTTTTT     1020
TTCTTTGTGT TTTAGGGTGA AACTTAAAAA AAAAATTCTG CCCCCACCTA GATCATATTT     1080
AAAGATCTTT TAGAAGTGAG AGAAAAAGGT CCTACGAAAA CGGAATAATA AAAAGCATTT     1140
GGTGCCTATT TGAAGTACAG CATAAGGGAA TCCCTTGTAT ATGCGAACAG TTATTGTTTG     1200
ATTATGTAAA AGTAATAGTA AAATGCTTAC AGGGAAACCT GCAGAGTAGT TAGAGAATAT     1260
GTATGCCTGC AATATGGGAC CAAATTAGAG GAGACTTTTT TTTTTCATGT TATGAGCTAG     1320
CACATACACC CCCTTGTAGT ATAATTTCAA GGAAGTGTGT ACGCCATTTA TCGATGATTA     1380
GATTGCAAAG CAATGAACTC AAGAAGGAAT TGAAATAAGG AGGGACATGA TGGGGAAGGA     1440
GTACAAAACA ATCTCTCAAC ATGATTGAAC CATTTGGGAT GGAGAAGCAC CTTTGCTCTC     1500
AGCCACCTGT TACTAAGTCA GGAGTGTAGT TGGATCTCTA CATTAATGTC CTCTTGCTGT     1560
CTACAGTAGC TGCTACCTAA AAAAAGATGT TTTATTTTGC CAGTTGGACA CAGGTGATTG     1620
GCTCCTGGGT TTCATGTTCT GTGACATCCT GCTTCTTCTT CCAAATGCAG TTCATTGCAG     1680
ACACCACCAT ATTGCTATCT AATGGGGAAA TGTAGCTATG GGCCATAACC AAAACTCACA     1740
TGAAACGGAG GCAGATGGAG ACCAAGGGTG GGATCCAGAA TGGAGTCTTT TCTGTTATTG     1800
TATTTAAAGG GGTAATGTGG CCTTGGCATT TCTTCTTAGA AAAAACTAA TTTTGGTGTC     1860
TGATTGGCAT GTCTGGTTCA CAGTTTAGCA TTGTTATAAA CCATTCCATT CGAAAAGCAC     1920
TTTGAAAAAT TGTTCCCGAG CGATAGATGG GATGGTTTAT GCAGGAATTC     1970

```

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 289 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Glu Leu Leu Cys His Glu Val Asp Pro Val Arg Arg Ala Val Arg
 1             5             10             15
Asp Arg Asn Leu Leu Arg Asp Asp Arg Val Leu Gln Asn Leu Leu Thr
          20             25             30

```

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Ile Glu Glu Arg Tyr Leu Pro Gln Cys Ser Tyr Phe Lys Cys Val Gln  
                   35                                  40                                  45  
 Lys Asp Ile Gln Pro Tyr Met Arg Arg Met Val Ala Thr Trp Met Leu  
                   50                                  55                                  60  
 Glu Val Cys Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu Ala  
                   65                                  70                                  75                                  80  
 Met Asn Tyr Leu Asp Arg Phe Leu Ala Gly Val Pro Thr Pro Lys Ser  
                                   85                                  90                                  95  
 His Leu Gln Leu Leu Gly Ala Val Cys Met Phe Leu Ala Ser Lys Leu  
                                   100                                  105                                  110  
 Lys Glu Thr Ser Pro Leu Thr Ala Glu Lys Leu Cys Ile Tyr Thr Asp  
                                   115                                  120                                  125  
 Asn Ser Ile Lys Pro Gln Glu Leu Leu Glu Trp Glu Leu Val Val Leu  
                   130                                  135                                  140  
 Gly Lys Leu Lys Trp Asn Leu Ala Ala Val Thr Pro His Asp Phe Ile  
                   145                                  150                                  155                                  160  
 Glu His Ile Leu Arg Lys Leu Pro Gln Gln Arg Glu Lys Leu Ser Leu  
                                   165                                  170                                  175  
 Ile Arg Lys His Ala Gln Thr Phe Ile Ala Leu Cys Ala Thr Asp Phe  
                                   180                                  185                                  190  
 Lys Phe Ala Met Tyr Pro Pro Ser Met Ile Ala Thr Gly Ser Val Gly  
                   195                                  200                                  205  
 Ala Ala Ile Cys Gly Leu Gln Gln Asp Glu Glu Val Ser Ser Leu Thr  
                   210                                  215                                  220  
 Cys Asp Ala Leu Thr Glu Leu Leu Ala Lys Ile Thr Asn Thr Asp Val  
                   225                                  230                                  235                                  240  
 Asp Cys Leu Lys Ala Cys Gln Glu Gln Ile Glu Ala Val Leu Leu Asn  
                                   245                                  250                                  255  
 Ser Leu Gln Gln Tyr Arg Gln Asp Gln Arg Asp Gly Ser Lys Ser Glu  
                                   260                                  265                                  270  
 Asp Glu Leu Asp Gln Ala Ser Thr Pro Thr Asp Val Arg Asp Ile Asp  
                   275                                  280                                  285  
 Leu

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1926 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCCGAT CCCAGCCCG CCCGCCGCG CTCTCCGGCC CGTCGCCTGC CTTGGGACTC

60

GCGAGCCCGC	ACTCCCGCCC	TGCCTGTTCC	CTGCCCGAGT	ATGGAGCTGC	TGTGTTGCGA	120
AGGCACCCGG	CACGCGCCCC	GGGCCGGGCC	GGACCCGCGG	CTGCTGGGGG	ACCAGCGTGT	180
CCTGCAGAGC	CTGCTCCGCC	TGGAGGAGCG	CTACGTACCC	CGCGCCTCCT	ACTTCCAGTG	240
CGTGCAGCGG	GAGATCAAGC	CGCACATGCG	GAAGATGCTG	GCTTACTGGA	TGCTGGAGGT	300
ATGTGAGGAG	CAGCGCTGTG	AGGAGGAAGT	CTTCCCCCTG	GCCATGAACT	ACCTGGATCG	360
CTACCTGTCT	TGCGTCCCCA	CCCGAAAGGC	GCAGTTGCAG	CTCCTGGGTG	CGGTCTGCAT	420
GGCCCCTGAC	CATCGAAAAA	CTGTGCATCT	ACACCGACCA	CGCTGTCGCC	AGTTGCGGGA	480
CTGGGAGGTG	CTGGTCCTAG	GGAAGCTCAA	GTGGGACCTG	GCTGCTGTGA	TTGCACATGA	540
TTTCCTGGCC	TTCATTCTGC	ACCGGCTCTC	TCTGCCCCGT	GACCGACAGG	CCTTGGTCAA	600
AAAGCATGCC	CAGACCTTTT	TGGCCCTCTG	TGCTACAGAT	TATACCTTTG	CCATGTACCC	660
GCCATCCATG	ATCGCCACGG	GCAGCATTGG	GGCTGCAGTG	CAAGGCCTGG	GTGCCTGCTC	720
CATGTCCGGG	GATGAGCTCA	CAGAGCTGCT	GGCAGGGATC	ACTGGCACTG	AAGTGGACTG	780
CCTGCGGGCC	TGTCAGGAGC	AGATCGAAGC	TGCACTCAGG	GAGAGCCTCA	GGGAAGCCGC	840
TCAGACCAGC	TCCAGCCCAG	CGCCCCAAGC	CCCCCGGGGC	TCCAGCAGCC	AAGGGCCCAG	900
CCAGACCAGC	ACTCTTACAG	ATGTCACAGC	CATACACCTG	TAGCCCTGGA	GAGGCCCTCT	960
GGAGTGGCCA	CTAAGCAGAG	GAGGGGCCGC	TGCACCCACC	TCCCTGCCTC	CAGGAACCAC	1020
ACCACATCTA	AGCCTGAAGG	GGCGTCTGTT	CCCCCTTCAC	AAAGCCCAAG	GGATCTGGTC	1080
CTACCCATCC	CCGCAGTGTG	CACTAAGGGG	CCCGGCCAGC	CATGTCTGCA	TTTCGGTGCC	1140
TAGTCAAGCT	CCTCCTCCCT	GCATCTGACC	AGCAGCGCCT	TTCCCAACTC	TAGCTGGGGG	1200
TGGGCCAGGC	TGATGGGACA	GAATTGGATA	CATACACCAG	CATTCTTTTT	GAACGCCCCC	1260
CCCCACCCCT	GGGGGCTCTC	ATGTTTTCAA	CTGCCAAAAT	GCTCTAGTGC	CTTCTAAAGG	1320
TGTTGTCCCT	TCTAGGGTTA	TTGCATTGGG	ATTGGGGTCC	CTCTAAAATT	TAATGCATGA	1380
TAGACACATA	TGAGGGGGAA	TAGTCTAGAT	GGCTCCTCTC	AGTACTTTGG	AGGCCCCCTAT	1440
GTAGTCCTGG	CTGACAGCTG	CTCCTAGAGG	GAGGGGCCTA	GGCTCAGCCA	GAGAAGCTAT	1500
AAATTCCTCT	TTGCTTTGCT	TTCTGCTCAG	CTTCTCCTGT	GTGATTGACA	GCTTTGCTGC	1560
TGAAGGCTCA	TTTAATTTA	TTAATTGCTT	TGAGCACAAC	TTTAAGAGGA	CGTAATGGGG	1620
TCCTGGCCAT	CCCACAAGTG	GTGGTAACCC	TGGTGGTTGC	TGTTTTCTCTC	CCTTCTGCTA	1680
CTGGCAAAAG	GATCTTTGTG	GCCAAGGAGC	TGCTATAGCC	TGGGGTGGGG	TCATGCCCTC	1740
CTCTCCCAT	GTCCCTCTGC	CCCATCCTCC	AGCAGGGAAA	ATGCAGCAGG	GATGCCCTGG	1800
AGGTGCTGAG	CCCCTGTCTA	GAGAGGGAGG	CAAGCCTGTT	GACACAGGTC	TTTCCTAAGG	1860
CTGCAAGGTT	TAGGCTGGTG	GCCCAGGACC	ATCATCTTAC	TGTAATAAAG	ATGATTGTGG	1920
GAATTC						1926

(2) INFORMATION FOR SEQ ID NO:6:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

[illegible]



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## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 819 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Leu Cys Cys Glu Val Glu Thr Ile Arg Arg Ala Tyr Pro Asp Ala  
 1 5 10 15  
 Asn Leu Leu Asn Asp Arg Val Leu Arg Ala Met Leu Lys Ala Glu Glu  
 20 25 30  
 Thr Cys Ala Pro Ser Val Ser Tyr Phe Lys Cys Val Gln Lys Glu Val  
 35 40 45  
 Leu Pro Ser Met Arg Lys Ile Val Ala Thr Trp Met Leu Glu Val Cys  
 50 55 60  
 Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr  
 65 70 75 80  
 Leu Asp Arg Phe Leu Ser Leu Glu Pro Val Lys Lys Ser Arg Leu Gln  
 85 90 95  
 Leu Leu Gly Ala Thr Cys Met Phe Ser Ile Val Leu Glu Asp Glu Lys  
 100 105 110  
 Pro Val Ser Val Asn Glu Val Pro Asp Tyr His Glu Asp Ile His Thr  
 115 120 125  
 Tyr Leu Arg Glu Met Glu Val Lys Cys Lys Pro Lys Val Gly Tyr Met  
 130 135 140  
 Lys Lys Gln Pro Asp Ile Thr Asn Ser Met Arg Ala Ile Leu Val Asp  
 145 150 155 160  
 Trp Leu Val Glu Val Gly Glu Glu Tyr Lys Leu Gln Asn Glu Thr Leu  
 165 170 175  
 His Leu Ala Val Asn Tyr Ile Asp Arg Phe Leu Ser Ser Met Ser Val  
 180 185 190  
 Leu Arg Gly Lys Leu Gln Leu Val Gly Thr Ala Ala Met Leu Lys Glu  
 195 200 205  
 Leu Pro Pro Arg Asn Asp Arg Gln Arg Phe Leu Glu Val Val Gln Tyr  
 210 215 220  
 Gln Met Asp Ile Leu Glu Tyr Phe Arg Glu Ser Glu Lys Lys His Arg  
 225 230 235 240  
 Pro Lys Pro Arg Tyr Met Arg Arg Gln Lys Asp Ile Ser His Asn Met  
 245 250 255  
 Arg Ser Ile Leu Ile Asp Trp Leu Val Glu Val Ser Glu Glu Tyr Lys  
 260 265 270  
 Leu Asp Thr Glu Thr Leu Tyr Leu Ser Val Phe Tyr Leu Asp Arg Phe  
 275 280 285

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Leu Ser Gln Met Ala Val Val Arg Ser Lys Leu Gln Leu Val Gly Thr  
 290 295 300  
 Ala Ala Met Tyr Val Asn Asp Val Asp Ala Glu Asp Gly Ala Asp Pro  
 305 310 315 320  
 Asn Leu Cys Ser Glu Tyr Val Lys Asp Ile Tyr Ala Tyr Leu Arg Gln  
 325 330 335  
 Leu Glu Glu Glu Gln Ala Val Arg Pro Lys Tyr Leu Leu Gly Arg Glu  
 340 345 350  
 Val Thr Gly Asn Met Arg Ala Ile Leu Ile Asp Trp Leu Val Gln Val  
 355 360 365  
 Gln Met Lys Phe Arg Leu Leu Gln Glu Thr Met Tyr Met Thr Val Ser  
 370 375 380  
 Ile Ile Asp Arg Phe Met Gln Asn Asn Cys Val Pro Lys Lys Met Leu  
 385 390 395 400  
 Gln Leu Val Gly Val Thr Ala Met Phe Trp Asp Asp Leu Asp Ala Glu  
 405 410 415  
 Asp Trp Ala Asp Pro Leu Met Val Ser Glu Tyr Val Val Asp Ile Phe  
 420 425 430  
 Glu Tyr Leu Asn Glu Leu Glu Ile Glu Thr Met Pro Ser Pro Thr Tyr  
 435 440 445  
 Met Asp Arg Gln Lys Glu Leu Ala Trp Lys Met Arg Gly Ile Leu Thr  
 450 455 460  
 Asp Trp Leu Ile Glu Val His Ser Arg Phe Arg Leu Leu Pro Glu Thr  
 465 470 475 480  
 Leu Phe Leu Ala Val Asn Ile Ile Asp Arg Phe Leu Ser Leu Arg Val  
 485 490 495  
 Cys Ser Leu Asn Lys Leu Gln Leu Val Gly Ile Ala Ala Leu Phe Ile  
 500 505 510  
 Glu Leu Ser Asn Ala Glu Leu Leu Thr His Tyr Glu Thr Ile Gln Glu  
 515 520 525  
 Tyr His Glu Glu Ile Ser Gln Asn Val Leu Val Gln Ser Ser Lys Thr  
 530 535 540  
 Lys Pro Asp Ile Lys Leu Ile Asp Gln Gln Pro Glu Met Asn Pro His  
 545 550 555 560  
 Gln Thr Arg Glu Ala Ile Val Thr Phe Leu Tyr Gln Leu Ser Val Met  
 565 570 575  
 Thr Arg Val Ser Asn Gly Ile Phe Phe His Ser Val Arg Phe Tyr Asp  
 580 585 590  
 Arg Tyr Cys Ser Lys Arg Val Val Leu Lys Asp Gln Ala Lys Leu Val  
 595 600 605  
 Val Gly Thr Cys Leu Trp Pro Asn Leu Val Lys Arg Glu Leu Gln Ala  
 610 615 620  
 His His Ser Ala Ile Ser Glu Tyr Asn Asn Asp Gln Leu Asp His Tyr  
 625 630 635 640

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 100 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu	Ala	Ser	Lys	Phe	Glu	Glu	Ile	Tyr	Pro	Pro	Glu	Val	Ala	Glu	Phe
1				5					10					15	
Val	Tyr	Ile	Thr	Val	Asp	Thr	Tyr	Thr	Lys	Lys	Gln	Val	Leu	Arg	Met
			20					25					30		
Glu	His	Leu	Val	Leu	Lys	Val	Leu	Thr	Phe	Asp	Leu	Ala	Ala	Pro	Thr
		35					40					45			
Val	Asn	Gln	Phe	Leu	Thr	Gln	Tyr	Phe	Leu	His	Gln	Gln	Asn	Cys	Lys
	50					55					60				
Val	Glu	Ser	Leu	Ala	Met	Phe	Leu	Gly	Glu	Leu	Ser	Leu	Ile	Asp	Ala
65					70					75					80
Asp	Pro	Tyr	Leu	Lys	Tyr	Leu	Pro	Ser	Val	Ile	Ala	Gly	Ala	Ala	Phe
				85					90					95	

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His Leu Ala Leu  
100

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ile Ala Ala Lys Tyr Glu Glu Ile Tyr Pro Pro Glu Val Gly Glu Phe  
1 5 10 15  
Val Phe Leu Thr Asp Asp Ser Tyr Thr Lys Ala Gln Val Leu Arg Met  
20 25 30  
Glu Gln Val Ile Leu Lys Ile Leu Ser Phe Asp Leu Cys Thr Pro Thr  
35 40 45  
Ala Tyr Val Phe Ile Asn Thr Tyr Ala Val Leu Cys Asp Met Pro Glu  
50 55 60  
Lys Leu Lys Tyr Met Thr Leu Tyr Ile Ser Glu Leu Ser Leu Met Glu  
65 70 75 80  
Gly Glu Thr Tyr Leu Gln Tyr Leu Pro Ser Leu Met Ser Ser Ala Ser  
85 90 95  
Val Ala Leu Ala Arg  
100

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 100 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ile Ala Ser Lys Tyr Glu Glu Met Tyr Pro Pro Glu Ile Gly Asp Phe  
1 5 10 15  
Ala Phe Val Thr Asp Asn Thr Tyr Thr Lys His Gln Ile Arg Gln Met  
20 25 30  
Glu Met Lys Ile Leu Arg Ala Leu Asn Phe Gly Leu Gly Arg Pro Leu  
35 40 45  
Pro Leu His Phe Leu Arg Arg Ala Ser Lys Ile Gly Glu Val Asp Val  
50 55 60  
Glu Gln His Thr Leu Ala Lys Tyr Leu Met Glu Leu Thr Met Leu Asp  
65 70 75 80  
Tyr Asp Met Val His Phe Pro Pro Ser Gln Ile Ala Ala Gly Ala Phe  
85 90 95

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Cys Leu Ala Leu  
100

## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 100 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile	Ala	Ser	Lys	Tyr	Glu	Glu	Val	Met	Cys	Pro	Ser	Val	Gln	Asn	Phe
1				5					10					15	
Val	Tyr	Met	Ala	Asp	Gly	Gly	Tyr	Asp	Glu	Glu	Glu	Ile	Leu	Gln	Ala
			20					25					30		
Glu	Arg	Tyr	Ile	Leu	Arg	Val	Leu	Glu	Phe	Asn	Leu	Ala	Tyr	Pro	Asn
			35				40					45			
Pro	Met	Asn	Phe	Leu	Arg	Arg	Ile	Ser	Lys	Ala	Asp	Phe	Tyr	Asp	Ile
			50			55					60				
Gln	Thr	Arg	Thr	Val	Ala	Lys	Tyr	Leu	Val	Glu	Ile	Gly	Leu	Leu	Asp
65					70				75					80	
His	Lys	Leu	Leu	Pro	Tyr	Pro	Pro	Ser	Gln	Gln	Cys	Ala	Ala	Ala	Met
				85					90					95	
Tyr	Leu	Ala	Arg												
															100

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 51 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu	Ala	Ala	Lys	Thr	Trp	Gly	Arg	Leu	Ser	Glu	Leu	Val	His	Tyr	Cys
1				5					10					15	
Gly	Gly	Ser	Asp	Leu	Phe	Asp	Glu	Ser	Met	Phe	Ile	Gln	Met	Glu	Arg
			20					25					30		
His	Ile	Leu	Asp	Thr	Leu	Asn	Trp	Asp	Val	Tyr	Glu	Pro	Met	Ile	Asn
			35				40					45			
Asp	Tyr	Ile													
															50

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 51 amino acids  
 (B) TYPE: amino acid

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(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ile	Ser	Ser	Lys	Phe	Trp	Asp	Arg	Met	Ala	Thr	Leu	Lys	Val	Leu	Gln
1				5					10					15	
Asn	Leu	Cys	Cys	Asn	Gln	Tyr	Ser	Ile	Lys	Gln	Phe	Thr	Thr	Met	Glu
		20					25						30		
Met	His	Leu	Phe	Lys	Ser	Leu	Asp	Trp	Ser	Ile	Ser	Ala	Thr	Phe	Asp
		35					40					45			
Ser	Tyr	Ile													
		50													

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCCAAAAAC GTCTTT

16

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCCAAAAAC GTCTTTAAAA GAGAGAGAGA G

31

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 175 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCCAAAAAC GTCTTTAAAA GAGAGAGAGA GAAAAAATA TAGTATTCC CAAAACTGT

60

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CTTTAAAGA GAGAGAGAGA AAAAAAATA GTATTCCTAA AACTGTCTT TAAAAGAGAG 120

AGAGAGAAAA AAAAAATAGT ATTTGCATAA CCCTGAGCGG TGGGGGAGGA GGGTT 175

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGCATAACCC TGAGCGGTGG GGGAGGAGGG TT 32

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGCATAACCC TGAGCGGTGG GGGAGGAGGG TT 32

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 295 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Glu His Gln Leu Leu Cys Cys Glu Val Glu Thr Ile Arg Arg Ala  
1 5 10 15

Tyr Pro Asp Ala Asn Leu Leu Asn Asp Arg Val Leu Arg Ala Met Leu  
20 25 30

Lys Ala Glu Glu Thr Cys Ala Pro Ser Val Ser Tyr Phe Lys Cys Val  
35 40 45

Gln Lys Glu Val Leu Pro Ser Met Arg Lys Ile Val Ala Thr Trp Met  
50 55 60

Leu Glu Val Cys Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu  
65 70 75 80

Ala Met Asn Tyr Leu Asp Arg Phe Leu Ser Leu Glu Pro Val Lys Lys  
85 90 95

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Ser Arg Leu Gln Leu Leu Gly Ala Thr Cys Met Phe Val Ala Ser Lys  
 100 105 110  
 Met Lys Glu Thr Ile Pro Leu Thr Ala Glu Lys Leu Cys Ile Tyr Thr  
 115 120 125  
 Asp Gly Ser Ile Arg Pro Glu Leu Leu Gln Met Glu Leu Leu Leu  
 130 135 140  
 Val Asn Lys Leu Lys Trp Asn Leu Ala Ala Met Thr Pro His Asp Phe  
 145 150 155 160  
 Ile Glu His Phe Leu Ser Lys Met Pro Glu Ala Glu Glu Asn Lys Gln  
 165 170 175  
 Ile Ile Arg Lys His Ala Gln Thr Phe Val Ala Leu Cys Ala Thr Asp  
 180 185 190  
 Val Lys Phe Ile Ser Asn Pro Pro Ser Met Val Ala Ala Gly Ser Val  
 195 200 205  
 Val Ala Ala Val Lys Gly Leu Asn Leu Arg Ser Pro Asn Asn Phe Leu  
 210 215 220  
 Ser Tyr Tyr Arg Leu Thr Arg Phe Leu Ser Arg Val Ile Lys Cys Asp  
 225 230 235 240  
 Pro Asp Cys Leu Arg Ala Cys Gln Glu Gln Ile Glu Ala Leu Leu Glu  
 245 250 255  
 Ser Ser Leu Arg Gln Ala Gln Gln Asn Met Asp Pro Lys Ala Ala Glu  
 260 265 270  
 Glu Glu Glu Glu Glu Glu Glu Glu Val Asp Leu Ala Cys Thr Pro Thr  
 275 280 285  
 Asp Val Arg Asp Val Asp Ile  
 290 295

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 295 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Glu Asn Gln Leu Leu Cys Cys Glu Val Glu Thr Ile Arg Arg Ala  
 1 5 10 15  
 Tyr Pro Asp Thr Asn Leu Leu Asn Asp Arg Val Leu Arg Ala Met Leu  
 20 25 30  
 Lys Thr Glu Glu Thr Cys Ala Pro Ser Val Ser Tyr Phe Lys Cys Val  
 35 40 45  
 Gln Lys Glu Ile Val Pro Ser Met Arg Lys Ile Val Ala Thr Trp Met  
 50 55 60  
 Leu Glu Val Cys Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu  
 65 70 75 80



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Ala Met Asn Tyr Leu Asp Arg Phe Leu Ser Leu Glu Pro Leu Lys Lys  
85 90 95

Ser Arg Leu Gln Leu Leu Gly Ala Thr Cys Met Phe Val Ala Ser Lys  
100 105 110

Met Lys Glu Thr Ile Pro Leu Thr Ala Glu Lys Leu Cys Ile Tyr Thr  
115 120 125

Asp Asn Ser Ile Arg Pro Glu Glu Leu Leu Gln Met Glu Leu Leu Leu  
130 135 140

Val Asn Lys Leu Lys Trp Asn Leu Ala Ala Met Thr Pro His Asp Phe  
145 150 155 160

Ile Glu His Phe Leu Ser Lys Met Pro Asp Ala Glu Glu Asn Lys Gln  
165 170 175

Ile Ile Arg Lys His Ala Gln Thr Phe Val Ala Leu Cys Ala Thr Asp  
180 185 190

Val Lys Phe Ile Ser Asn Pro Pro Ser Met Val Ala Ala Gly Ser Met  
195 200 205

Val Ala Ala Met Gln Gly Leu Asn Leu Gly Ser Pro Asn Asn Phe Leu  
210 215 220

Ser Arg Tyr Arg Thr Thr His Phe Leu Ser Arg Val Ile Lys Cys Asp  
225 230 235 240

Pro Asp Cys Leu Arg Ala Cys Gln Glu Gln Ile Glu Ala Leu Leu Glu  
245 250 255

Ser Ser Leu Arg Gln Ala Gln Gln Asn Met Asp Pro Lys Ala Thr Glu  
260 265 270

Glu Glu Gly Glu Val Glu Glu Glu Ala Gly Leu Ala Cys Thr Pro Thr  
275 280 285

Asp Val Arg Asp Val Asp Ile  
290 295

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 189 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Glu Leu Leu Cys His Glu Val Asp Pro Val Arg Arg Ala Val Arg  
1 5 10 15

Asp Arg Asn Leu Leu Arg Asp Asp Arg Val Leu Gln Asn Leu Leu Thr  
20 25 30

Ile Glu Glu Arg Tyr Leu Pro Gln Cys Ser Tyr Phe Lys Cys Val Gln  
35 40 45

Lys Asp Ile Gln Pro Tyr Met Arg Arg Met Val Ala Thr Trp Met Leu  
50 55 60

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Glu Val Cys Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu Ala  
 65 70 75 80  
 Met Asn Tyr Leu Asp Arg Phe Leu Ala Gly Val Pro Thr Pro Lys Ser  
 85 90 95  
 His Pro Pro Ser Met Ile Ala Thr Gly Ser Val Gly Ala Ala Ile Cys  
 100 105 110  
 Gly Leu Lys Gln Asp Glu Glu Val Ser Ser Leu Thr Cys Asp Ala Leu  
 115 120 125  
 Thr Glu Leu Leu Ala Lys Ile Thr Asn Thr Asp Val Asp Cys Leu Lys  
 130 135 140  
 Ala Cys Gln Glu Gln Ile Glu Ala Val Leu Leu Asn Ser Leu Gln Gln  
 145 150 155 160  
 Tyr Arg Gln Asp Gln Arg Asp Gly Ser Lys Ser Glu Asp Glu Leu Asp  
 165 170 175  
 Gln Ala Ser Thr Pro Thr Asp Val Arg Asp Ile Asp Leu  
 180 185

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 236 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Arg Arg Met Val Ala Thr Trp Met Leu Glu Val Cys Glu Glu Gln  
 1 5 10 15  
 Lys Cys Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr Leu Asp Arg  
 20 25 30  
 Phe Leu Ala Gly Val Pro Thr Pro Lys Thr His Leu Gln Leu Leu Gly  
 35 40 45  
 Ala Val Cys Met Phe Leu Ala Ser Lys Leu Lys Glu Thr Ile Pro Leu  
 50 55 60  
 Thr Ala Glu Lys Leu Cys Ile Tyr Thr Asp Asn Ser Val Lys Pro Gln  
 65 70 75 80  
 Glu Leu Leu Glu Trp Glu Leu Val Val Leu Gly Lys Leu Lys Trp Asn  
 85 90 95  
 Leu Ala Ala Val Thr Pro His Asp Phe Ile Glu His Ile Leu Arg Lys  
 100 105 110  
 Leu Pro Gln Gln Lys Glu Lys Leu Ser Leu Ile Arg Lys His Ala Gln  
 115 120 125  
 Thr Phe Ile Ala Leu Cys Ala Thr Asp Phe Lys Phe Ala Met Tyr Pro  
 130 135 140  
 Pro Ser Met Ile Ala Thr Gly Ser Val Gly Ala Ala Ile Cys Gly Leu  
 145 150 155 160

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Gln Gln Asp Asp Glu Val Asn Thr Leu Thr Cys Asp Ala Leu Thr Glu  
                                   165                                  170                                  175

Leu Leu Ala Lys Ile Thr His Thr Asp Val Asp Cys Leu Lys Ala Cys  
                                   180                                  185                                  190

Gln Glu Gln Ile Glu Ala Leu Leu Leu Asn Ser Leu Gln Gln Phe Arg  
                                   195                                  200                                  205

Gln Glu Gln His Asn Ala Gly Ser Lys Ser Val Glu Asp Pro Asp Gln  
                                   210                                  215                                  220

Ala Thr Thr Pro Thr Asp Val Arg Asp Val Asp Leu  
                                   225                                  230                                  235

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 292 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Glu Leu Leu Cys Cys Glu Gly Thr Arg His Ala Pro Arg Ala Gly  
 1                                  5                                  10                                  15

Pro Asp Pro Arg Leu Leu Gly Asp Gln Arg Val Leu Gln Ser Leu Leu  
                                   20                                  25                                  30

Arg Leu Glu Glu Arg Tyr Val Pro Arg Ala Ser Tyr Phe Gln Cys Val  
                                   35                                  40                                  45

Gln Arg Glu Ile Lys Pro His Met Arg Lys Met Leu Ala Tyr Trp Met  
                                   50                                  55                                  60

Leu Glu Val Cys Glu Glu Gln Arg Cys Glu Glu Glu Val Phe Pro Leu  
                                   65                                  70                                  75                                  80

Ala Met Asn Tyr Leu Asp Arg Tyr Leu Ser Cys Val Pro Thr Arg Lys  
                                   85                                  90                                  95

Ala Gln Leu Gln Leu Leu Gly Ala Val Cys Met Leu Leu Ala Ser Lys  
                                   100                                  105                                  110

Leu Arg Glu Thr Thr Pro Leu Thr Ile Glu Lys Leu Cys Ile Tyr Thr  
                                   115                                  120                                  125

Asp His Ala Val Ser Pro Arg Gln Leu Arg Asp Trp Glu Val Leu Val  
                                   130                                  135                                  140

Leu Gly Lys Leu Lys Trp Asp Leu Ala Ala Val Ile Ala His Asp Phe  
                                   145                                  150                                  155                                  160

Leu Ala Phe Ile Leu His Arg Leu Ser Leu Pro Arg Asp Arg Gln Ala  
                                   165                                  170                                  175

Leu Val Lys Lys His Ala Gln Thr Phe Leu Ala Leu Cys Ala Thr Asp  
                                   180                                  185                                  190

Tyr Thr Phe Ala Met Tyr Pro Pro Ser Met Ile Ala Thr Gly Ser Ile  
                                   195                                  200                                  205

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Gly Ala Ala Val Gln Gly Leu Gly Ala Cys Ser Met Ser Gly Asp Glu  
 210 215 220

Leu Thr Glu Leu Leu Ala Gly Ile Thr Gly Thr Glu Val Asp Cys Leu  
 225 230 235 240

Arg Ala Cys Gln Glu Gln Ile Glu Ala Ala Leu Arg Glu Ser Leu Arg  
 245 250 255

Glu Ala Ala Gln Thr Ser Ser Ser Pro Ala Pro Lys Ala Pro Arg Gly  
 260 265 270

Ser Ser Ser Gln Gly Pro Ser Gln Thr Ser Thr Pro Thr Asp Val Thr  
 275 280 285

Ala Ile His Leu  
 290

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 237 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Arg Lys Met Leu Ala Tyr Trp Met Leu Glu Val Cys Glu Glu Gln  
 1 5 10 15

Arg Cys Glu Glu Asp Val Phe Pro Leu Ala Met Asn Tyr Leu Asp Arg  
 20 25 30

Tyr Leu Ser Cys Val Pro Thr Arg Lys Ala Gln Leu Gln Leu Leu Gly  
 35 40 45

Thr Val Cys Ile Leu Leu Ala Ser Lys Leu Arg Glu Thr Thr Pro Leu  
 50 55 60

Thr Ile Glu Lys Leu Cys Ile Tyr Thr Asp Gln Ala Val Ala Pro Trp  
 65 70 75 80

Gln Leu Arg Glu Trp Glu Val Leu Val Leu Gly Lys Leu Lys Trp Asp  
 85 90 95

Leu Ala Ala Val Ile Ala His Asp Phe Leu Ala Leu Ile Leu His Arg  
 100 105 110

Leu Ser Leu Pro Ser Asp Arg Gln Ala Leu Val Lys Lys His Ala Gln  
 115 120 125

Thr Phe Leu Ala Leu Cys Ala Thr Asp Tyr Thr Phe Ala Met Tyr Pro  
 130 135 140

Pro Ser Met Ile Ala Thr Gly Ser Ile Gly Ala Ala Val Ile Gly Leu  
 145 150 155 160

Gly Ala Cys Ser Met Ser Ala Asp Glu Leu Thr Glu Leu Leu Ala Gly  
 165 170 175

Ile Thr Gly Thr Glu Val Asp Cys Leu Arg Ala Cys Gln Glu Gln Ile  
 180 185 190

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(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 106 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met	Arg	Ala	Ile	Leu	Val	Asp	Trp	Leu	Val	Glu	Val	Gly	Glu	Glu	Tyr
1				5					10					15	
Lys	Leu	Gln	Asn	Glu	Thr	Leu	His	Leu	Ala	Val	Asn	Tyr	Ile	Asp	Arg
			20					25					30		
Phe	Leu	Ser	Ser	Met	Ser	Val	Leu	Arg	Gly	Lys	Leu	Gln	Leu	Val	Gly
		35					40					45			
Thr	Ala	Ala	Met	Leu	Leu	Ala	Ser	Lys	Phe	Glu	Glu	Ile	Tyr	Pro	Pro
	50					55					60				
Glu	Val	Ala	Glu	Phe	Val	Tyr	Ile	Thr	Asp	Asp	Thr	Tyr	Thr	Lys	Lys
65					70					75					80
Gln	Val	Leu	Arg	Met	Glu	His	Leu	Val	Leu	Lys	Val	Leu	Thr	Phe	Asp
				85					90					95	
Leu	Ala	Ala	Pro	Thr	Val	Asn	Gln	Phe	Leu						
			100					105							

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 116 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met	Arg	Ala	Ile	Leu	Val	Asp	Trp	Leu	Val	Met	Arg	Ala	Ile	Leu	Ile
1				5					10					15	
Asp	Trp	Leu	Val	Gln	Val	Gln	Met	Lys	Phe	Arg	Leu	Leu	Gln	Glu	Thr
			20					25					30		
Met	Tyr	Met	Thr	Val	Ser	Ile	Ile	Asp	Arg	Phe	Met	Gln	Asn	Asn	Cys
		35					40					45			
Val	Pro	Lys	Lys	Met	Leu	Gln	Leu	Val	Gly	Val	Thr	Ala	Met	Phe	Ile
	50					55					60				

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Ala Ser Lys Tyr Glu Glu Met Tyr Pro Pro Glu Ile Gly Asp Phe Ala  
 65 70 75 80  
 Phe Val Thr Asp Asn Thr Tyr Thr Lys His Gln Ile Arg Gln Met Glu  
 85 90 95  
 Met Lys Ile Leu Arg Ala Leu Asn Phe Gly Leu Gly Arg Pro Leu Pro  
 100 105 110  
 Leu His Phe Leu  
 115

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Arg Ala Ile Leu Val Asp Trp Leu Val Gln Val His Ser Lys Phe  
 1 5 10 15  
 Arg Leu Leu Gln Glu Thr Leu Tyr Met Cys Val Gly Ile Met Asp Arg  
 20 25 30  
 Phe Leu Gln Val Gln Pro Val Ser Arg Lys Lys Leu Gln Leu Val Gly  
 35 40 45  
 Ile Thr Ala Leu Leu Leu Ala Ser Lys Tyr Glu Glu Met Phe Ser Pro  
 50 55 60  
 Asn Ile Glu Asp Phe Val Tyr Ile Thr Asp Asn Ala Tyr Thr Ser Ser  
 65 70 75 80  
 Gln Ile Arg Glu Met Glu Thr Leu Ile Leu Lys Glu Leu Lys Phe Glu  
 85 90 95  
 Leu Gly Arg Pro Leu Pro Leu His Phe Leu  
 100 105

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 105 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Leu Gln Ile Phe Phe Thr Asn Val Ile Gln Ala Leu Gly Glu His Leu  
 1 5 10 15  
 Lys Leu Arg Gln Gln Val Ile Ala Thr Ala Thr Val Tyr Phe Lys Arg  
 20 25 30  
 Phe Tyr Ala Arg Tyr Ser Leu Lys Ser Ile Asp Pro Val Leu Met Ala  
 35 40 45

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Pro Thr Cys Val Phe Leu Ala Ser Lys Val Glu Glu Ile Leu Lys Thr  
 50 55 60

Arg Phe Ser Tyr Ala Phe Pro Lys Glu Phe Pro Tyr Arg Met Asn His  
 65 70 75 80

Ile Leu Glu Cys Glu Phe Tyr Leu Leu Glu Leu Met Asp Cys Cys Leu  
 85 90 95

Ile Val Tyr His Pro Tyr Arg Pro Leu  
 100 105

## (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 104 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Met Arg Ala Ile Leu Leu Asp Trp Leu Met Glu Val Cys Glu Val Tyr  
 1 5 10 15

Lys Leu His Arg Glu Thr Phe Tyr Leu Ala Gln Asp Phe Phe Asp Arg  
 20 25 30

Tyr Met Ala Glu Asn Val Val Lys Thr Leu Leu Gln Leu Ile Gly Ile  
 35 40 45

Ser Ser Leu Phe Ile Ala Ala Lys Leu Glu Glu Ile Tyr Pro Pro Lys  
 50 55 60

Leu His Gln Phe Ala Tyr Val Thr Asp Gly Ala Cys Ser Gly Asp Glu  
 65 70 75 80

Ile Leu Thr Met Glu Leu Met Ile Met Lys Ala Leu Lys Trp Arg Leu  
 85 90 95

Ser Pro Leu Thr Ile Val Ser Trp  
 100

## (2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1462 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TGATCAAGTT GAACTCAAT ATTAACCCTC ATAGACTGTG ATCCCTATGT TGCTGCCTTC 60

CCTCGTTTCT ATTGCTCTTT GGCCCCAACC CAAATAAGGT TCCTTGGGAC AACTAAAGA 120

AGGAGGTGGA GTTCGAAGGG GAGGAGAGAT GTGAGCGAGG CAGGCAGGGA AGCTCTGCTC 180

GCCCACTGCC CAATCCTCAC CTCTCTTCTC CTCCACCTTC TGTCTCTGCC CTCACCTCTC 240

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CTCTGAAAAC CCCCTATTGA GCCAAAGGAA GGAGATGAGG GGAATGCTTT TGCCTTCCCC      300
CTCCAAAACA AAAACAAAAA CAAACACACT TTTCCAGTCC AGAGAAAGCA GGGGAGTGAG      360
GGGTCACAGA GCTGGCCATG CAGCTGCTGG GCTGTGAGGT AGACCCGGTC CTCAGAGCCA      420
CGAGGGACTG CAACCTACTC CAAGTTGACC GTGTCCTGAA GAACCTGCTT GCTATCAAGA      480
AGCGCTACCT TCAGTAATGC TCCTACTTCA AGTGTGTGCA GAAGGCCATC CAGCCGTACA      540
TGCACAGGAT GGTGCCACTT CTGATGGTGG CCATTTGATT GGTGCCACTT CTGATGGTGG      600
CCAACATGAT TGAACCATTT GGGATGGAAA AGCACCTTTA CTCTCAGCCA CCTGTTAACT      660
AATGCTGGAG GTCTGTGAGG AACAGAAGTG TGAAGAAAAG GTTTTCCCTC TGGCCACGAT      720
TTACCTGGAC TGTTCCTTCG CCAGGATCCC AACTTCAAAG TCCCATCTGC AACTCCTGGG      780
TGCTGTCTGC ATGTTCTCTG CCTCCAGGCT CAAAGAGTCC AGCCCACTGA CTGCCAAAAA      840
GCTGTGCATT TATACCGACA ACTCCATCAA GCCTCAGGAG CTGCTGGAGT GGGAACTGGT      900
GGTGTGGGGA AAGTTGAAGT GGAACCTGGC AGCTGTCACG CCTCATGACT TCATTTAGTA      960
CATCTTGCCAC AAGCTGCCCC AGCAGCGGGA GAAGCTGTCT CCAATCTGCA AGCAAGTCCA     1020
GAACTTCAAT GCTCTGTATG CAATGTACCC GCCATCAATG GTTGCAACTG GAAGTGTAGG     1080
AGCAGCTATC TGTGGACTTC AGCAACATGA GGAAGTGAGC TCACTCCCTT GCAATGCCCT     1140
GACTGAGCTG CTGGCAAAGA TCACCAACAC AGATGTGGAT TGTCTCAAAA GCCAACC GGG      1200
AGCATATTGA GGTGGTCTTC CTCAACAGCC TGCAGCAGTG CCATCAGGAC CAGCAGGACA     1260
GATCCAAGTC AGAGGATGAA CTGGGCCAAG CAGCACCCCT ATAGACCTGT GAGATATCGA     1320
CCTGTGAGGA TGGCAGTCCA GCTGAGAGGC GCATTCTATA TCTGCTGTCT CCTTCTTTCT     1380
GGTTATGTTT TGTTCTTTGT ATCTTAGGGC GAAACTTAAA AAAAAAACC TCTGCCCCCA     1440
CATAGTTCGT GTTTAAAGAT CT                                             1462

```

## (2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 269 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```

Met  Gln  Leu  Leu  Gly  Cys  Glu  Val  Asp  Pro  Val  Leu  Arg  Ala  Thr  Arg
 1              5              10              15

Asp  Cys  Asn  Leu  Leu  Gln  Val  Asp  Arg  Val  Leu  Lys  Asn  Leu  Leu  Ala
          20              25              30

Ile  Lys  Lys  Arg  Tyr  Leu  Gln  Cys  Ser  Tyr  Phe  Lys  Cys  Val  Gln  Lys
          35              40              45

Ala  Ile  Gln  Pro  Tyr  Met  His  Arg  Met  Val  Pro  Leu  Leu  Met  Val  Met
50              55              60

```



Leu 65	Glu	Val	Cys	Glu 70	Gln	Lys	Cys	Glu 75	Lys	Val	Phe	Pro	Leu 80		
Ala	Thr	Ile	Tyr	Leu 85	Asp	Cys	Phe	Phe	Ala 90	Arg	Ile	Pro	Thr	Ser	Lys
Ser	His	Leu	Gln 100	Leu	Leu	Gly	Ala	Val 105	Cys	Met	Phe	Leu	Ala 110	Ser	Arg
Leu	Lys	Glu 115	Ser	Ser	Pro	Leu	Thr 120	Ala	Lys	Lys	Leu	Cys 125	Ile	Tyr	Thr
Asp	Asn 130	Ser	Ile	Lys	Pro	Gln 135	Glu	Leu	Leu	Glu	Gln 140	Glu	Leu	Val	Val
Leu 145	Gly	Lys	Leu	Lys	Trp 150	Asn	Leu	Ala	Ala	Val 155	Thr	Pro	His	Asp	Phe 160
Ile	Tyr	Ile	Leu 165	His	Lys	Leu	Pro	Gln 170	Gln	Arg	Glu	Lys	Leu	Ser 175	Ala
Met	Tyr	Pro	Pro 180	Ser	Met	Val	Ala	Thr 185	Gly	Ser	Val	Gly	Ala 190	Ala	Ile
Cys	Gly	Leu 195	Gln	Gln	His	Glu	Glu 200	Val	Ser	Ser	Leu	Pro 205	Cys	Asn	Ala
Leu 210	Thr	Glu	Leu	Leu	Ala	Lys 215	Ile	Thr	Asn	Thr	Asp 220	Val	Asp	Cys	Leu
Lys 225	Ala	Asn	Arg	Glu	His 230	Ile	Glu	Val	Val	Phe 235	Leu	Asn	Ser	Leu	Gln 240
Gln	Cys	His	Gln 245	Asp	Gln	Gln	Asp	Arg	Ser 250	Lys	Ser	Glu	Asp	Glu	Leu 255
Gly	Gln	Ala	Ser 260	Thr	Pro	Ile	Asp 265	Leu	Asp	Ile	Asp	Leu			

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1901 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAGCTTCCAG	ATTAGAAAAG	AAAAAATAAA	ACTATCTTTA	TTTGCAGATG	ACATGATCGG	60
TCCATTCTCA	TGCTGCTTAT	AAAGACATAC	CCAAGACTGG	ATAATTTATA	AAGGAAAGAG	120
GTTTGGCTCA	CAGTTCCCCA	TGGGTGGAGA	GGCCTCACAA	TCATGGCGAA	AGAGCAAGGA	180
GCATCTCACA	TGGCAGCAGG	CAAGAAAAGA	ATGAGAGCCA	CGCCAGAGGG	AAACCCCTTA	240
TAAAATCATC	AGATCTCGAG	AGACTTATTC	ACTGTCAGGA	GAACAGTATG	GAGGAAACGC	300
CCTTATGATT	CAATTATCTC	GCACTGTGTT	CCTCCCACAA	CACATGGGAA	TTATGGGAGC	360
TACAATTCAA	GATGAGATTT	GGGTGGAGAC	ACAGCCAAAC	CATATCAATC	TTTTTTTCT	420

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TATTCTTTTT	TTTTTTTTTT	TTTTTTTTGA	GATGGAGTCC	CACTCTGTTA	TCTAGGCTGG	480
AGTGCAGTGG	TGTGTGATCT	TGGCTCACTG	CAACCTCAGC	CTCCCAGGTT	CAAGCGATTG	540
TCCTGCCTCA	GACTCCTGAA	TAGCTGAAAT	TACAGGCACC	TGCCACTACG	CCTGGCAAAT	600
ATTTTTTGTT	TGTTTGTTTG	TTTGTTTGTT	TGTTTTGAGA	CAGAGTCTCT	CTCTGTCGCC	660
CAGGCTGGAG	TGCAGTGGGC	GCGATCTCAG	CTCACTGCAA	ACTCTGCTCC	CGGGTTCAAG	720
CCATTCTCCT	GCCTCAGCTC	CCAAGTAGCT	GGGACTACAG	GCGCCACCA	CCACCATGCC	780
AGGCTAATTT	TTTGTATTTT	TAGTAGAGAC	AGGGTTTCAC	CGTGTTAGCC	AGGATGGTCT	840
CAATCTCCTG	ACCTCGTGAT	CCGCCCACCT	CGGCCTCCCA	AAGTGCTGGG	ATTACAGGCG	900
TGAGCCACTA	TGCCCAACCG	TATCAATCTT	GTATATAGAA	AAACCTAAGG	AATCTACAAA	960
AAAACCCTAT	TATAACTAAT	ATAATAATAA	TCTGCAAAGT	TGTAGACTAT	GAGATCAATA	1020
TACAAAAATT	AACTCAATTT	CTTTACATGT	ACAATGAATA	ACCCCAAAC	AAAAC TGGGA	1080
ATATAATTCT	ATTTTAAATA	GTATCACAAA	GAATGACAAT	ACTTAGAAAC	AAATGATGGG	1140
CGCTAGCTTG	CACTCCCGCC	CTGCCTGTGC	GCTGCCCGAG	TGTGGAGCTG	CTATGCTGCG	1200
AAGGCTCGAG	GACCCGCAGA	CGCCAGGGGA	TCAGCGCGTC	CTGCAGAGCT	TGCTCCCTT	1260
GGAGTAGCGC	TGCGTGCACT	GCGCCTACTT	CCAGTGCGTG	CAAAGGGAGA	GCAAGCCGCA	1320
CATGCGGAAG	ATGCTGGTTT	ACTGGATGCT	GGAGGTGTGT	GAGGAGCAGT	GCTGTGAGGA	1380
GGAGCAGTGC	TGTAAGGAGG	AAGTCTTTCC	CCTGGCCATG	AACCACCTGC	ATGCTACCTG	1440
TCCTACGTCC	CCACCCACCC	GAAAGGCACA	GTTGCAGCTC	TTGGTTGCGG	TCTCCATGCG	1500
GCTGGCCTCC	AAGCTGCGTA	AGACTGGGCC	CATGACCATT	GAGAAAATGT	GCATCTACAC	1560
CGACCACGCT	GTCTCTCCCT	GCCAGTTGCG	GGACTGGGAG	GTGATGGTCC	TGGGGAAGCT	1620
CAAATGGGAC	CTGGCCGCTG	TGATTGCTCA	TGACTTCTTG	GCCCTCATTC	TGCACCGACA	1680
CAGATAACCA	TATGTGATAT	ATATCAATAC	AATGGAATAT	GGCCTGGCAT	GCTGGCTTAC	1740
GCTGTAATCC	TGCAC TTTGG	GAGGCCAAAG	TGGAGGATCA	CTTGAGCCGA	GGAGTTCAAG	1800
GCCAGCCTGG	GCACAAAGTG	AGACTCCTTC	TAAAAAATA	AAATAAAATA	AAAAATAAAA	1860
ACAATGTAAT	ATTATTCAGC	CATAGAAAGG	AATAAAGTAC	T		1901

## (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 215 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Trp	Ala	Leu	Ala	Cys	Thr	Pro	Ala	Leu	Pro	Val	Arg	Cys	Pro	Ser	Val
1				5					10					15	

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Glu Leu Leu Cys Cys Glu Gly Ser Arg Asp Pro Gln Thr Pro Gly Asp  
                     20                    25                    30  
 Gln Arg Val Leu Gln Ser Leu Leu Pro Leu Glu Arg Cys Val His Cys  
                     35                    40                    45  
 Ala Tyr Phe Gln Cys Val Gln Arg Glu Ser Lys Pro His Met Arg Lys  
                     50                    55                    60  
 Met Leu Val Tyr Trp Met Leu Glu Val Cys Glu Glu Cys Cys Glu Glu  
                     65                    70                    75                    80  
 Glu Cys Cys Lys Glu Glu Val Phe Pro Leu Ala Met Asn His Leu His  
                     85                    90                    95  
 Ala Thr Cys Pro Thr Ser Pro Pro Thr Arg Lys Ala Gln Leu Gln Leu  
                     100                    105                    110  
 Leu Val Ala Val Ser Met Arg Leu Ala Ser Lys Leu Arg Lys Thr Gly  
                     115                    120                    125  
 Pro Met Thr Ile Glu Lys Met Cys Ile Tyr Thr Asp His Ala Val Ser  
                     130                    135                    140  
 Pro Cys Gln Leu Arg Asp Trp Glu Val Met Val Leu Gly Lys Leu Lys  
                     145                    150                    155                    160  
 Trp Asp Leu Ala Ala Val Ile Ala His Asp Phe Leu Ala Leu Ile Leu  
                     165                    170                    175  
 His Arg Arg Gln Ala Leu Val Lys Lys His Ala Gln Ile Phe Leu Ala  
                     180                    185                    190  
 Val Cys Ala Thr Asp Tyr Thr Phe Ala Met Tyr Pro Pro Ser Ser Cys  
                     195                    200                    205  
 Glu Asn Asn Pro Asn Ala Cys  
                     210                    215

## (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1317 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GAGCTCGATC AGTACACTCG TTTGTTTAAT TGATAATTGT CCTGAATTAT GCCGGCTCCT	60
GCAGCCCCCT CACGCTCAGC AATTCAGTCC CAGGGCAAAT TCTAAAGGTG AAGGGACGTC	120
TACACCCCCA ACAAACCAA TTAGGAACCT TCGGTGGGTC TTGTCCCAGG CAGAGGGGAC	180
TAATATTTCC AGCAATTTAA TTTCTTTTTT AATTAAAAAA AATGAGTCAG AATGGAGATC	240
ACTGTTTCTC AGCTTTCCAT TCAGAGGTGT GTTTCTCCCG GTTAAATTGC CGGCACGGGA	300
AGGGAGGGGG TGCAGTTGGG GACCCCCGCA AGGACCGACT GGTCAAGGTA GGAAGGCAGC	360
CCGAAGAGTC TCCAGGCTAG AAGGACAAGA TGAAGGAAAT GCTGGCCACC ATCTTGGGCT	420

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GCTGCTGGAA TTTTCGGGCA TTTATTTTAT TTTATTTTTT GAGCGAGCGC ATGCTAAGCT	480
GAAATCCCTT TAACTTTTAG GTTACCCCTT GGGCATTGTC AACGACGCCC CTGTGCGCCG	540
GAATGAAACT TGCACAGGGG TTGTGTGCCC GGTCTCTCCC GTCCTTGCAT GCTAAATTAG	600
TTCTTGCAAT TTACACGTGT TAATGAAAAT GAAAGAAGAT GCAGTCGCTG AGATTCTTTG	660
GCCGTCTGTC CGCCCGTGGG TGCCCTCGTG GCGTTCTTGG AAATGCGCCC ATTCTGCCGG	720
CTTGATATG GGGTGTGCCC GCGCCCCAGT CACCCCTTCT CGTGGTCTCC CCAGGCTGCG	780
TGCTGGCCGG CCTTCCTAGT TGTCCCTAC TGCAGAGCCA CCTCCACCTC ACCCCCTAAA	840
TCCCGGGACC CACTCGAGGC GGACGGGCCC CCTGCACCCC TCTCGGCGGG GAGAAAGGCT	900
GCAGCGGGGC GATTTCGATT TCTATGAAAA CCGGACTACA GGGGCAACTG CCCGCAGGGC	960
AGCGCGGCGC CTCAGGGATG GCTTTTCGTC TGCCCTCTGC TGCTCCCGGC GTTCTGCCCC	1020
CGCCCCCTCC CCCTGCGCCC GCCCCGCCC CCCTCCCGCT CCCATTCTCT GCCGGGCTTT	1080
GATCTTTGCT TAACAACAGT AACGTCACAC GGACTACAGG GGAGTTTTGT TGAAGTTGCA	1140
AAGTCCTGGA GCCTCCAGAG GGCTGTCGGC GCAGTAGCAG CGAGCAGCAG AGTCCGCACG	1200
CTCCGGCGAG GGGCAGAAGA GCGCGAGGGA GCGCGGGGCA GCAGAAGCGA GAGCCGAGCG	1260
CGGACCCAGC CAGGACCCAC AGCCCTCCCC AGCTGCCAG GAAGAGCCCC AGCCATG	1317

## (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1624 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAGCTCGAGC CACGCCATGC CCGCTGCACG TGCCAGCTTG GCCAGCACAT CAGGGCGCTG	60
GTCTCTCCCC TTCCTCCTGG AGTGAAATAC ACCAAAGGGC GCGGTGGGGG TGGGGGTGA	120
CGGGAGGAAG GAGGTGAAGA AACGCCACCA GATCGTATCT CCTGTAAAGA CAGCCTTGAC	180
TCAAGGATGC GTTAGAGCAC GTGTCAGGGC CGACCGTGCT GCGGGCGACT TCACCGCAGT	240
CGGCTCCCAG GGAGAAAGCC TGGCGAGTGA GGCGCGAAAC CGGAGGGGTC GGCGAGGATG	300
CGGGCGAAGG ACCGAGCGTG GAGGCCTCAT GCTCCGGGGA AAGGAAGGGG TGGTGGTGT	360
TGCGCAGGGG GAGCGAGGGG GAGCCGGACC TAATCCCTTC ACTCGCCCCC TTCCCTCCCG	420
GGCCATTTCC TAGAAAGCTG CATCGGTGTG GCCACGCTCA GCGCAGACAC CTCGGGCGGC	480
TTGTCAGCAG ATGCAGGGGC GAGGAAGCGG GTTTTTCCTG CGTGGCCGCT GGCGCGGGGG	540
AACCGCTGGG AGCCCTGCCC CCGGCCTGCG GCGGCCCTAG ACGCTGCACC GCGTCGCCCC	600
ACGGCGCCCG AAGAGCCCCC AGAAACACGA TGGTTTCTGC TCGAGGATCA CATTCTATCC	660
CTCCAGAGAA GCACCCCCCT TCCTTCCTAA TACCACCTC TCCCTCCCTC TTCTTCCTCT	720

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GCACACACTC TGCAGGGGGG GGCAGAAGGG ACGTTGTTCT GGTCCCTTTA ATCGGGGCTT	780
TCGAAACAGC TTCGAAGTTA TCAGGAACAC AGACTTCAGG GACATGACCT TTATCTCTGG	840
GTATGCGAGG TTGCTATTTT CTAAAATCAC CCCCTCCCTT ATTTTTCACCT TAAGGGACCT	900
ATTTCTAAAT TGTCTGAGGT CACCCCATCT TCAGATAATC TACCCTACAT TCCTGGATCT	960
TAAATACAAG GGCAGGAGGA TTAGGATCCG TTTTGAAGA AGCCAAAGTT GGAGGGTCGT	1020
ATTTTGGCGT GCTACACCTA CAGAATGAGT GAAATTAGAG GGCAGAAATA GGAGTCGGTA	1080
GTTTTTTGTG GGTGCCCTG TCCGGGCCCC TGGCATGCAG GCTTGGATGG AGGGAGAGGG	1140
GTTGGGGGTT GCGGGGGACC GCGTTTGAAG TTGGGTCGGG CCAGCTGCTG TTCTCCTTAA	1200
TAACGAGAGG GGAAAAGGAG GGAGGGAGGG AGAGATTGAA AGGAGAGGGG GAGGACCGGG	1260
AGGGGAGGAA AGGGGAGGAG GAACCAGAGC GGGGAGCGCG GGGAGAGGGA GGAGAGCTAA	1320
CTGCCCAGCC AGCTTCGGTC ACGCTTCAGA GCGGAGAAGA GCGAGCAGGG GAGAGCGAGA	1380
CCAGTTTTTA GGGGAGGACC GGTGCGAGTG AGGCAGCCCC TAGGCTCTGC TCGCCCACCA	1440
CCCAATCCTC GCCTCCCTTC TGCTCCACCT TCTCTCTCTG CCCTCACCTC TCCCCGAAA	1500
ACCCCCTATT TAGCCAAAGG AAGGAGGTCA GGAACGCTC TCCCCTCCCC TTCCAAAAAA	1560
CAAAAACAGA AAAACCTTT TCCAGGCCGG GGAAAGCAGG AGGGAGAGGG CGCGGGCTGC	1620
CATG	1624

## (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1317 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAGCTCGATC AGTACACTCG TTTGTTTAAT TGATAATTGT CCTGAATTAT GCCGGCTCCT	60
GCAGCCCCCT CACGCTCACG AATTCAGTCC CAGGGCAAAT TCTAAAGGTG AAGGGACGTC	120
TACACCCCCA ACAAAACCAA TTAGGAACCT TCGGTGGGTC TTGTCCCAGG CAGAGGGGAC	180
TAATATTTCC AGCAATTTAA TTTCTTTTTT AATTAAAAAA AATGAGTCAG AATGGAGATC	240
ACTGTTTCTC AGCTTTCCAT TCAGAGGTGT GTTCTCCCG GTTAAATTGC CGGCACGGGA	300
AGGGAGGGGG TGCAATTGGG GACCCCCGCA AGGACCGACT GGTCAAGGTA GGAAGGCAGC	360
CCGAAGAGTC TCCAGGCTAG AAGGACAAGA TGAAGGAAAT GCTGGCCACC ATCTTGGGCT	420
GCTGCTGGAA TTTTCGGGCA TTTATTTTAT TTTATTTTTT GAGCGAGCGC ATGCTAAGCT	480
GAAATCCCTT TAACTTTTAG GTTACCCCTT GGGCATTTGC AACGACGCCC CTGTGCGCCG	540
GAATGAAACT TGCACAGGGG TTGTGTGCCC GGTCTCCCC GTCTTGCAT GCTAAATTAG	600
TTCTTGCAAT TTACACGTGT TAATGAAAAT GAAAGAAGAT GCAGTCGCTG AGATTCTTTG	660

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GCCGTCTGTC CGCCCGTGGG TGCCCTCGTG GCGTTCTTGG AAATGCGCCC ATTCTGCCGG	720
CTTGATATG GGGTGTGCGC GCGCCCCAGT CACCCCTTCT CGTGGTCTCC CCAGGCTGCG	780
TGCTGGCCGG CCTTCCTAGT TGTCCCCTAC TGCAGAGCCA CCTCCACCTC ACCCCCTAAA	840
TCCCGGGACC CACTCGAGGC GGACGGGCCC CCTGCACCCC TCTCGGCGGG GAGAAAGGCT	900
GCAGCGGGGC GATTTGCATT TCTATGAAAA CCGGACTACA GGGGCAACTG CCCGCAGGGC	960
AGCGCGGCGC CTCAGGGATG GCTTTTCGTC TGCCCCCTCGC TGCTCCCGGC GTTCTGCCCC	1020
CGCCCCCTCC CCCTGCGCCC GCCCCGCCC CCCTCCCGCT CCCATTCTCT GCCGGGCTTT	1080
GATCTTTGCT TAACAACAGT AACGTCACAC GGACTACAGG GGAGTTTGT TGAAGTTGCA	1140
AAGTCCTGGA GCCTCCAGAG GGCTGTCGGC GCAGTAGCAG CGAGCAGCAG AGTCCGCACG	1200
CTCCGGCGAG GGGCAGAAGA GCGCGAGGGA GCGCGGGGCA GCAGAAGCGA GAGCCGAGCG	1260
CGGACCCAGC CAGGACCCAC AGCCCTCCCC AGCTGCCCAG GAAGAGCCCC AGCCATG	1317

## (2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TGGATGYTNG ARGNTNGYGA RGARCARAAR TGYGARGA	38
---	----

## (2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 13 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Trp	Met	Leu	Glu	Val	Cys	Glu	Glu	Gln	Lys	Cys	Glu	Glu
1				5					10			

## (2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 28 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

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GTNTTYCCNY TNGCNATGAA YTAYTNGA

28

## (2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Val	Phe	Pro	Leu	Ala	Met	Asn	Tyr	Leu	Asp
1				5				10	

## (2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

RTCNGTGTAD ATRCANARYT TYTC

24

## (2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 8 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Glu	Lys	Leu	Cys	Ile	Tyr	Thr	Asp
1				5			

WHAT IS CLAIMED IS:

1. Recombinant cyclin of mammalian origin which replaces a CLN-type protein essential for cell start in budding yeast.
- 5 2. Recombinant cyclin of Claim 1 which is D-type cyclin.
3. Recombinant cyclin of Claim 2 which is of human origin.
4. Recombinant D type cyclin of Claim 3 selected from the group consisting of: cyclin D1, cyclin D2 and cyclin D3.
- 10 5. Purified D-type cyclin of mammalian origin of approximate molecular weight 34 kD.
6. Purified D type cyclin of Claim 5 having the amino acid sequence of Figure 2, the amino acid sequence of Figure 3 or the amino acid sequence of Figure 4.
- 15 7. Purified D type cyclin of Claim 5 which is selected from the group consisting of: cyclin D1, cyclin D2 and cyclin D3.
8. Recombinant D-type cyclin of mammalian origin of approximate molecular weight 34 kD.
9. Recombinant D-type cyclin of Claim 8 having the amino  
20 acid sequence of Figure 2, the amino acid sequence of Figure 3 or the amino acid sequence of Figure 4.
10. Isolated DNA encoding D-type cyclin of mammalian origin of approximate molecular weight 34 kD.
- 25 11. Isolated DNA of Claim 10 having the nucleic acid sequence of Figure 2, the nucleic acid sequence of Figure 3 or the nucleic acid sequence of figure 4.



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12. Isolated DNA encoding a D-type cyclin protein which replaces a CLN-type protein essential for cell cycle start in budding yeast.
13. A DNA probe which hybridizes to at least a portion of  
5 a nucleic acid sequence selected from the group consisting of: the nucleic acid sequence of Figure 2, the nucleic acid sequence of Figure 3 and the nucleic acid sequence of Figure 4.
14. A DNA probe of Claim 13 which is labelled.
- 10 15. A labelled DNA probe of Claim 14 wherein the label is selected from the group consisting of: radioactive labels, fluorescent labels, enzymatic labels and binding pair members.
- 15 16. An antibody which specifically binds D-type cyclin of mammalian origin of approximate molecular weight 34 kD.
17. An antibody of Claim 16 which is a labelled monoclonal antibody.
18. A method of identifying DNA which replaces a gene essential for cell cycle start in yeast, comprising the  
20 steps of:
- a) providing mutant yeast cells in which the gene essential for cell cycle start is conditionally expressed;
  - b) introducing into mutant yeast cells of (a) a yeast vector which contain DNA to be assessed for its ability to  
25 replace a gene essential for cell cycle start in yeast and which expresses the DNA in the mutant yeast cells; and
  - c) selecting transformed mutant yeast cells produced in (b) on the basis of their ability to grow under conditions under which the gene essential for cell cycle  
30 start in the mutant yeast cells provided in (a) is not expressed, wherein ability to grow under the conditions of (c) is indicative of the presence in transformed mutant

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yeast cells of DNA which replaces a gene essential for cell cycle start.

19. The method of Claim 18 wherein the mutant yeast cells have inactive CLN1 and CLN2 genes and an altered CLN3 gene  
5 which is conditionally expressed from a glucose-repressible promoter; the yeast vector is pADNS and screening in (c) is carried out by assessing the ability of transformed mutant yeast produced in (b) to grow in the presence of glucose.

20. The method of Claim 19 wherein the DNA which replaces  
10 a gene essential for cell cycle start in yeast is a D-type cyclin.

21. The method of Claim 20 further comprising confirming that ability to grow in the presence of glucose is not the result of reversion by affirming stability of the yeast  
15 vector in transformed mutant yeast selected in (c).

22. A method of identifying DNA encoding cyclin which replaces a gene essential for cell cycle start in yeast, comprising the steps of:

a) providing mutant yeast cells in which the CLN1  
20 gene and the CLN2 gene are inactive and the CLN3 gene is conditionally expressed;

b) introducing into mutant yeast cells of (a) the yeast vector pADNS containing DNA to be assessed for its ability to replace the CLN3 gene, thereby producing  
25 transformed mutant yeast cells;

c) maintaining transformed mutant yeast cells produced in (b) on glucose-containing medium; and

d) selecting transformed mutant yeast cells produced in (b) on the basis of their ability to grow on glucose-  
30 containing medium.

23. The method of Claim 22 further comprising confirming the stability of the yeast vector pADNS in transformed mutant yeast cells selected in (d).

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24. The method of Claim 23 wherein the cyclin which replaces a gene essential for cell cycle start in yeast is a D-type cyclin.

25. A method of detecting DNA encoding a cyclin of  
5 mammalian origin in a cell, comprising the steps of:

- a) processing cells to render nucleic acid sequences present in the cells available for hybridization with complementary nucleic acid sequences;
  - b) combining the product of (a) with DNA encoding a  
10 D-type cyclin of mammalian origin or DNA complementary to DNA encoding a D-type cyclin of mammalian origin;
  - c) maintaining the product of (b) under conditions appropriate for hybridization of complementary nucleic acid sequences; and
  - 15 d) detecting hybridization of complementary nucleic acid sequences,
- wherein hybridization is indicative of the presence of DNA encoding a D-type cyclin of mammalian origin.

26. The method of Claim 25 wherein in (b) the product of  
20 (a) is combined with DNA selected from the group consisting of: DNA having the sequence of Figure 2; DNA complementary to the sequence of Figure 2; DNA having the sequence of Figure 3; and DNA complementary to the sequence of Figure 3.

27. The method of Claim 26 wherein the cyclin is a D-type  
25 cyclin.

28. The method of Claim 27 further comprising comparing hybridization detected in (d) with hybridization detected in appropriate control cells, wherein if hybridization detected in (d) is greater than hybridization in the control cells,  
30 it is indicative of increased levels of the DNA encoding the D-type cyclin of mammalian origin.

29. A method of detecting a D-type cyclin in a biological sample, comprising the steps of:

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a) providing a biological sample to be assessed for D-type cyclin level;

b) combining the biological sample with an antibody specific for a D-type cyclin; and

5 c) detecting binding of the antibody of (b) with a component of the biological sample, wherein binding is indicative of the presence of a D-type cyclin.

30. The method of Claim 29 wherein the antibody specific  
10 for a D-type cyclin is labelled.

31. A method of detecting amplification of a D-type cyclin in a biological sample, comprising the steps of:

a) providing a biological sample to be assessed for D-type cyclin level;

15 b) combining the biological sample with an antibody specific for a D-type cyclin;

c) determining the extent to which the antibody specific for a D-type cyclin binds to D-type cyclin in the biological sample; and

20 d) comparing the results of (c) with the extent to which the antibody specific for a D-type cyclin binds to D-type cyclin in an appropriate control, wherein greater binding of the antibody to D-type cyclin in the biological sample than in the appropriate control is  
25 indicative of amplification of the D-type cyclin.

32. The method of Claim 31 wherein the antibody specific for a D-type cyclin is labelled.

33. A method of detecting in a cell an increased level of a D-type cyclin of mammalian origin, comprising the steps  
30 of:

a) processing cells to be analyzed to render nucleic acids present in the cells available for hybridization with complementary nucleic acid sequences;

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- b) combining the product of (a) with DNA which hybridizes with DNA encoding a D-type cyclin of mammalian origin under the conditions used;
  - c) maintaining the combination of (b) under  
5 conditions appropriate for hybridization of complementary nucleic acid sequences;
  - d) detecting hybridization of complementary nucleic acid sequences; and
  - e) comparing hybridization detected in (d) with  
10 hybridization in appropriate control cells,  
wherein hybridization is indicative of the presence of a D-type cyclin of mammalian origin and greater hybridization in (d) than in the control cells is indicative of increased levels of the D-type cyclin of mammalian origin.
- 15 34. A method of inhibiting cell division comprising introducing into a cell a drug which interferes with formation in the cell of the protein kinase-D type cyclin complex essential for cell cycle start.
- 20 35. The method of Claim 34 wherein the drug is selected from the group consisting of:
- a) oligonucleotide sequences which bind DNA encoding D-type cyclins;
  - b) antibodies which specifically bind D-type cyclins;
  - c) agents which degrade D-type cyclins; and
  - 25 d) oligopeptides.
36. A method of interfering with activation in a cell of a protein kinase essential for cell cycle start, comprising introducing into the cell a drug selected from the group consisting of:
- 30 a) oligonucleotides which bind DNA encoding D-type cyclins;
  - b) peptides which bind the protein kinase essential for cell cycle start but do not activate it;
  - c) antibodies which specifically bind D-type cyclins;
  - 35 and

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- d) agents which degrade D-type cyclins.

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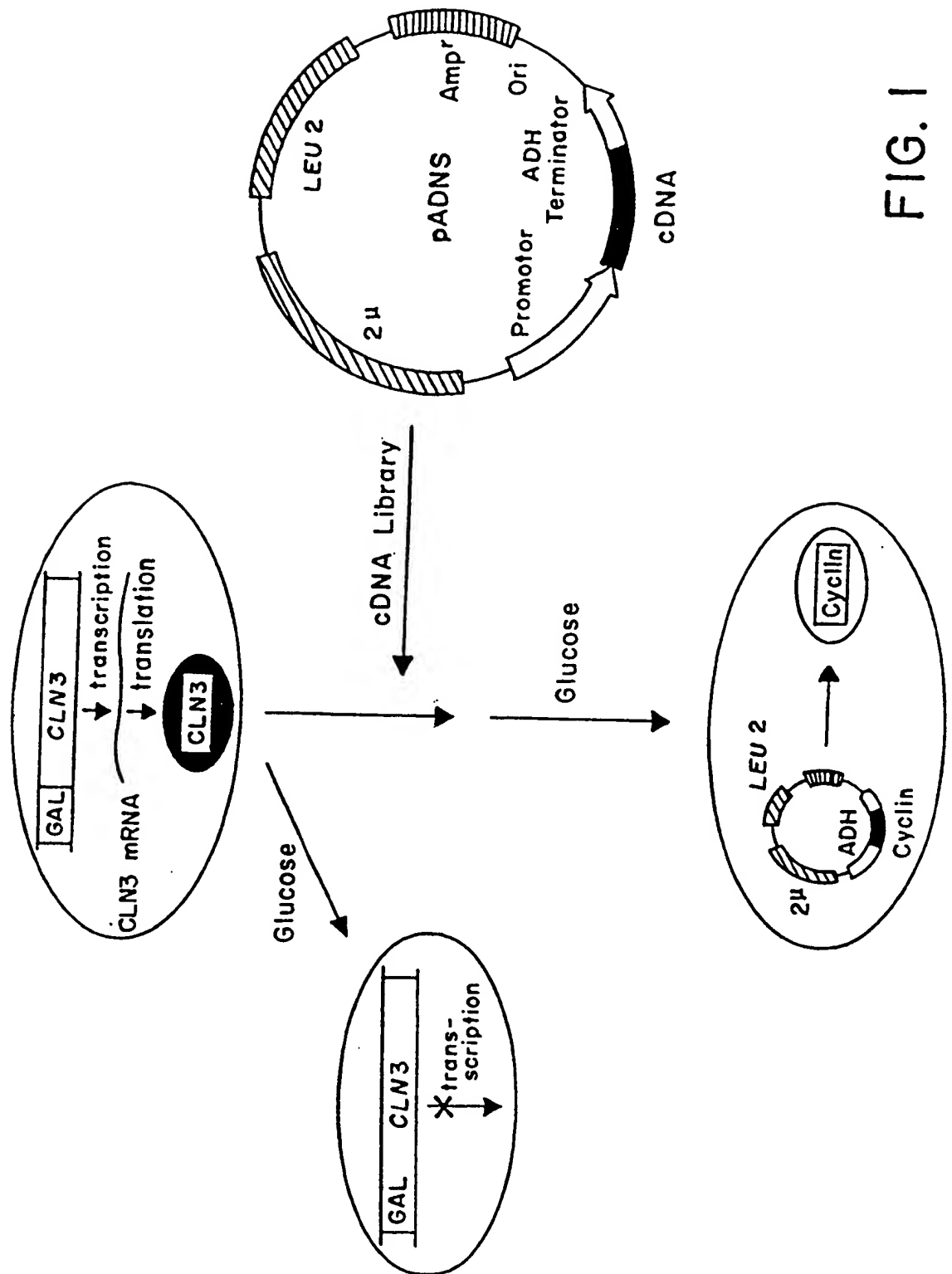


FIG. 1

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      . . . . .
GCAGTAGCAGCGAGCAGAGTCCGCGACGCTCCGGCGAGCGCCAGAACAGCGCGAGGGA
      . . . . .
GGCGGGGCGCAGCAGAAAGCGAGAGCGCGGAGCCAGGAGCCACAGCCCTCCCC      120

      . . . . .
AGTGCCCCAGGAAGAGCCCCAGCCCATGGAACACACAGCTCCTGTGTGCGGAAGTGGAAACC
      . . . . .
      M E H Q L L C C E V E T
      . . . . .
ATCCGCGCGCGGTACCCCGATGCCCAACCTCCTCAACGACCGGGTGTGCGGGCCCATGCTG      240
I R R A Y P D A N L L N D R V L R A M L      32

      . . . . .
AAGCGGAGGAGACCTGCGCGCCCTCGGTGTCTACTTCAAATGTGTGCAGAACGACGTC
      . . . . .
      K A E E T C A P S V S Y F K C V Q K E V
      . . . . .
CTCCCGTCCATGCCGAAGATCGTGGCCACCTGGATGTGGAGGTCTGCGAGGAACAGAAG      360
L P S M R K I V A T W M L E V C E E Q K      72

      . . . . .
TGGAGGAGGAGCTCTTCCCGCTGGCCCATGAACCTGGACCGGTTCTGTGCTGGAG
      . . . . .
      C E E E V F P L A M N Y L D R F L S L E
      . . . . .
CCCGTGAAAAGAGCGCGCTGCAGCTGCTGGGGGCCACTTGCAATGTTGCGTGGCCTCTAAG      480
P V K K S R L Q L L L G A T C M F V A S K      112

      . . . . .
ATGAAGGAGACCATCCCCCTGACGGCGGAGAGCTGTGCATCTACACCGACGCCCTCCATC
      . . . . .
      M K E T I P L T A E K L C I Y T D G S I
      . . . . .
CCCCCGAGGACCTGTGCAAAATGGAGCTGCTCCTGGTGAACAAGCTCAAGTGGAAACCTG      600
R P E E L L Q M E L L L V N K L K W N L      152

```

FIGURE 2



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GCCGCAATGACCCCGCAGGATTTCATTGAACACTTCTCCAAAATGACAGAGCGGGAG  
 A A M T P H D F I E H F L S K M P E A E  
 GAGAACAAACAGATCATCCGCGAAACACGCGCAGACCTTCGTTGCCTCTGTGTGCCACAGAT 720  
 E N K Q I I R K H A Q T F V A L C A T D 192  
 CTGAAGTTTCATTCCAAATCCGCGCCCTCCATGGTGGCAGCGGGACCGTGGTCGCCCGCAGTG  
 V K F I S N P P S M V A A G S V V A A V  
 AAGGCTGAACCTGAGGAGCCCCCAACAACCTTCCTGTCTGTACTACCGCCTCACACGCTTC 840  
 Q G L N L R S P N F L S Y R L T R F 232  
 CTCTCCAGAGTGATCAAGTGTGACCCAGACTGCCTCCGGGCCCTCCCGAGGAGCAGATCGAA  
 L S R V I K C D P D C L R A C Q E Q I E  
 GCCCTGCTGGAGTCAAGCCTGCGCCAGGCCACCAGAACATGGACCCCAAGGCCGCCGAG 960  
 A L L E S S L R Q A Q Q N M D P K A A E 272  
 GAGGAGGAAGAGGAGGAGGTGGACCTGGCTTGCACACCCACCGACGTCCCGGAC  
 E E E E E E V D L A C T P T D V R D  
 CTGGACATCTGAGGGCCCGAGGCGGGCCGCCACCCGCCAGCGGAGGGCGGAGC 1080  
 V D I \* (SEQ ID No. 2)  
 CGGCCCCAGGTGCTCCACATGACAGTCCCTCCTCCGGAGCATTTTGATACCAGAAGGG  
 AAACCTTCATTCTCCTTGTGTGGTTGTTTTTTCCTTGTCTCTTCCCCCTTCCATCTC 1200

FIGURE 2 (continued)

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· · · · ·  
TCACTTAACCAAAACAAAAGATTACCCAAA·CTGTCTTTAAAGAGAGAGAGAGAAAA·  
·  
AAAAAAA·AA· 1320

AAAAA 1325 (SEQ ID No. 1)

FIGURE 2 (continued)

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GAATCCCGCGGGCTTGGCCATGGAGCTGCTGTGCCACGAGGTGGACCCGGTCCGCAGG  
     M E L L C H E V D P V R R  
 GCGTGGCGGACCGCAACCTGCTCGGAGACCGCGTCTGCAGAACCTGCTCACCATC  
     A V R D R N L L R D D R V L Q N L L T I  
 120 33  
 GAGGAGCGCTACCTTCCGCAGTCTCCTACTTCAAGTCCGTGCAGAAGGACATCCAAACCC  
     E E R Y L P Q C S Y F K C V Q K D I Q P  
 TACATGCGCAGAATGGTGGCCACCTGGATGCTGGAGTCTGTGAGGAACAGAAGTGCCAA  
     Y M R R M V A T W M L E V C E E Q K C E  
 240 73  
 GAAGAGGTCTTCCCTCTGGCCCATGAATTACCTGGACCGTTTCTTGGCTGGGGTCCCGACT  
     E E V F P L A M N Y L D R F L A G V P T  
 CCGAAGTCCCATCTGCAACTCCTGGGTGCTGTGCTGCATGTTCTTGGCCTCCAAACTCAA  
     P K S H L Q L L G A V C M F L A S K L K  
 360 113  
 GAGACCAAGCCCTGACCGGAGAGAGCTGTGCATTACACCGACAACCTCCATCAAGCCT  
     E T S P L T A E K L C I Y T D N S I K P  
 CAGGAGCTGCTGGAGTGGGAACCTGGTGGTGGTGGGAAAGTTGAAGTGGAACTGGCAGCT  
     Q E L L E W E L V V L G K L K W N L A A  
 480 153

FIGURE 3

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GTCACCTCCTCATGACTTCAATTGAGCAGCATCTTGCGCAAGCTGCCCCAGCAGCGGGAGAAG  
 V T P H D F I E H I L R K L P Q Q R E K  
 CTGTCTCTGATCCGCAAGCATGCTCAGACCTTCAATTGCTCTGTGTGCCACCGACTTTAAG 600  
 L S L I R K H A Q T F I A L C A T D F K 193

TTTGCCATGTACCCACCGTCGATGATCGCAACTGGAAGTGTGGGAGCAGCCATCTGTGGG  
 F A M Y P P S M I A T G S V G A A I C G  
 CTCACGAGGATGAGGAAGTGAGCTCGCTCACTTGTGATGCCCTGACTGCTGGCT 720  
 L Q Q D E E V S S L T C D A L T E L A 234

AAGATCACCAACACAGACGTGGATTGTCTCAAAGCTTGCCAGGACCAGATTGAGGCGGTG  
 K I T N T D V D C L K A C Q E Q I E A V  
 CTCCTCAATAGCCTGCAGCAGTACCGTCAGGACCAACGTGACGGATCCAAGTCGGAGGAT 840  
 L L N S L Q Q Y R Q D Q R D G S K S E D 274

GAACTGGACCAAGCCAGCACCCCTACAGACGTGCGGGATATCGACCTGTGAGGATGCCAG  
 E L D Q A S T P T D V R D I D L \* 290 (SEQ ID No. 4)  
 TTGGGCGGAAAGAGAGAGACCGCGTCCATAATCTGGTCTCTTCTTCTTCTGGTGTGTTT 960  
 GTTCTTTGTGTTTAGGGTGAACTTAAAAAAAATTTCTGCCCCCCTAGATCATATT  
 TAAAGATCTTTTAGAAGTGAGAGAAAAAGGTCTCTACGAAAACGGAATAATAAAAGCATT 1080

FIGURE 3 (cont.)

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TGGTGCCCTATTGGAAGTACAGCATAAAGGGAATCCCTTGATATATGCGAACAGTTATTGTTT  
GATTATGTAAAGTAATAGTAAATGCTTACAGGGAACCTGCAGAGTAGTTAGAGAATA 1200

TGTATGCCCTGCAATATGGGACCAAAATTAGAGGAGACTTTTTCATGTTATGAGCTA  
GCACATACACCCCTTGTAGTATAATTCAAGGAACCTGTGTACGCCATTATCGATGATT 1320

AGATTGCCAAAGCAATGAACCTCAAGAAAGGAATTGAAATAAGGAGGGACATGATGGGGAAGG  
AGTACAAAACAATCTCTCAACATGATTGAACCATTTGGGATGGAGAAAGCACCTTTGCTCT 1440

CAGCCACCTGTTACTAAGTCAGGAGTGTAGTTGGATCTCTACATTAATGTCCCTTGCTG  
TCTACAGTAGCTGCTACCTAAATAAAGATGTTTATTTGCCAGTTGGACACAGGTGATT 1560

GGCTCCTGGGTTTCATGTTCTGTGACATCCTGCTTCTTCTTCCAAATGCAGTTCATTGCA  
GACACCACCATATTGCTATCTAATGGGGAATGTAGCTATGGGCCATAACCAAACTCAC 1680

ATGAAACGGAGGCAGATGGAGACCAAGGGTGGGATCCAGAAATGGAGTCTTTCTGTTATT  
GTATTTAAAGGGTAATGTGGCCCTTGGCATTCTTCTTAGAAAAAACTAATTTTGGTG 1800

CTGATTGGCATGTCTGGTTCACAGTTTAGCATTTGTATAAACCATTCCTCCATTCGAAAAGCA  
CTTTGAAAAATGTTCCCGAGCGATAGATGGGATGGTTTATGCAGGAATTC 1911 (SEQ ID No. 3)

FIGURE 3 (cont.)

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GAATTCGATCCCGAGCCCGCCCGCCCGCTCTCCGGCCCGCTCGCCTGCGCTGGGACTC  
 GCGAGCCCGCACTCCCGCCCTGCCCTGTTGCTGCCCGAGTAGGAGCTGCTGTGTTGCGA 120  
 M E L L C C E 7  
 AGGCACCCGGCAGCGCCCGCCCGCCCGCCCGGCTGCTGGGGACCGCGTGT  
 G T R H A P R A G P D P R L L G D Q R V  
 CCTGCAGAGCCTGCTCCCGCCTGGAGGAGCGCTACGTACCCCGCCCTCCTACTTCCAGTG 240  
 L Q S L L R L E E R Y V P R A S Y P Q C 47  
 CGTGCAGCGGAGATCAAGCGCACATGCGGAAGATGCTGGCTTACTGGATGCTGGAGGT  
 V Q R E I K P H M R K M L A Y W M L E V  
 ATGTGAGGAGCAGCGCTGTGAGGAGGAAGTCTTCCCCCTGGCCATGAACCTACCTGGATCG 360  
 C E E Q R C E E E V F P L A M N Y L D R 87  
 CTACCTGTCTTGGTCCCGACCCGAAAGCGCAGTTGCAGCTCCTGGGTGGTCTGCAT  
 Y L S C V P T R K A Q L Q L L G A V C M  
 GCTGTGGCCTCCAAGCTGCGGAGACCGCCCGCCCTGACCATCGAAAACTGTGCATCTA 480  
 L L A S K L R E T T P L T I E K L C I Y 127  
 CACCGACCGCTGTCTTCCCGCCAGTTGCGGGACTGGGAGGTGCTGCTCCTAGGAA  
 T D J A V S P R Q L R D W E V L V L G K  
 GCTCAAGTGGGACCTGGCTGTGTGATTGCACATGATTCTCGGCCCTTCATTCTGCACCG 600  
 L K W D L A A V I A H D F L A F I L H R 167

FIGURE 4

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GCTCTCTGCCCCGTGACCGACAGGCCCTTGTTCAAAAAGCATGCCAGACCTTTTGGC  
 L S L P R D R Q A L V K K H A Q T F L A  
 CCTCTGTGTACAGATTATACCTTTGCCATGTACCCGCCATCCATGATGCCACGGGCAG 720  
 L C A T, D Y T F A M Y P P S M I A T G S 207  
 CATTTGGGCTGCAGTGCAAGGCCCTGGGTGCCTGCTCCATGTCCGGGATGAGCTCACAGA  
 I G A A V Q G L G A C S M S G D E L T E  
 GCTGTGCGCAGGGATCACTGGCAGTGAAGTGGACTGCTGCCGGCCTGTACGAGCAGAT 840  
 L L A G I T G T E V D C L R A C Q E Q I 247  
 CGAAGCTGCACTCAGGGAGAGCCCTCAGGGAAGCCGCTCAGACCAGCTCCAGCCAGCGCC  
 E A A L R E S L R E A A Q T S S P A P  
 CAAGCCCCCGGGCTCCAGCAGCCCAAGGGCCAGCCAGCAGCACTCTTACAGATGT 960  
 K A P R G S S S Q G P S Q T S T P T D V 287  
 CACAGCCATACCTGTAGCCCTGGAGAGGCCCTCTGGAGTGGCCACTAAGCAGAGGAGG  
 T A I H L \* 292 (SEQ ID No. 6)  
 GGCCGCTGACCCACCTCCCTGCTCCAGGAACCAACACACATCTAAGCCTGAAGGGCG 1080  
 TCTGTTCCTTCACAAAGCCCAAGGATCTGGTCTTACCCATCCCCGAGTGTGCACT  
 AAGGGCCCCGGCCAGCCATGTCTGCATTTCCGGTGGCTAGTCAAGCTCCTCCTCCCTGCAT 1200  
 CTGACCAGCAGCGCCTTTCCCAACTCTAGCTGGGGTGGCCAGGCTGATGGGACAGAAAT  
 TGGATACATACACAGCATTCCTTTTGAAGCCCCCCCCCAGCCCTGGGGCTCTCATGT 1320

FIGURE 4 (continued)

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TTTCAACTGCCAAAATGCTCTAGTGCCTTCTAAAGGTGTTGTCCCTTCTAGGGTTATTGC  
ATTGGATTGGGTCCTCTAAAATTTAATGCATGATAGACACATAGAGGGGAATAGT 1440

CTAGATGGCTCCTCTCAGTACTTTGGAGGCCCTATGTAGTCCCTGGCTGACAGCTGCTCC  
TAGAGGAGGGCCTAGGCTCAGCCAGAGAGCTATAAATCCCTTTGCTTTGCTTTCT 1560

GCTCAGCTTCTCCTGTGTGATTGACAGCTTTGCTGCTGAAGGCTCATTTTAAATTATTA  
TTGCTTTGAGCACAACTTTAAGAGGACGTAATGGGTCCTGGCCATCCCCACAAGTGGTG 1680

TAAACCTGGTGGTTGCTGTTTTCTCCTCCCTCTGCTACTGGCAAAGGATCTTTGTGGCCA  
AGGAGCTGTATAGCCTGGGTGGGTGCATGCCCTCCTCTCCCATTTGTCCCTCTGCCCCA 1800

TCCTCCAGCAGGGAATGCAGCAGGGATGCCCTGGAGGTGCTGAGCCCTGTCTAGAGA  
GGGAGGCAAGCCTGTTGACACAGGTCTTTCCTAAGGCTGCAAGGTTTAGGCTGGTGCCC 1920

AGGACCATCATCCTACTGTAAATAAAGATGATTGTGGGAATTC 1962 (SEQ ID No. 5)

FIGURE 4 (continued)



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CYCD1-Hs	QLCCEVETIRRAYPDANLLNDRVLRAMLKAEETCAPSVSYFKCVQKEVLP SMRKIVATWMLVEVCEEQKCEEEVFPLAMNYLDRFLSLEPVPKKSRLQLLGATCMF
CYCA-Hs	SIVLEDEKPVSVNEVPDYHEDIHTYLR-EMEVKCKPKVGYMCKQP-DITN SMRAILLVDNLVEVGEEYKQLQNETLHLAVNYIDRFLSSMSVLRGKQLVGTAML
CYCA-Dm	KELPBRNDRQRFLEVQQOMDILEYFR-ESEKKHRPKPRYMRQK-DISH NMRSILLIDWLVEVSEYKLDTTETLYLSVYLDLDRFLSQMAVVRSKLQLVGTAAAY
CYCB1-Hs	VNDVDAEDGADPNLCSEYVKDIYAYLR-QLEEEQAVRPKYLLGR--EVTG NMRAILLIDWLQVQMKFRLLQETMYMTVSIIDRFMQNNCVPPKMLQLVGVGTAMF
CDC13-Sp	WDDLDAEDWADPLMVSEYVVDIFEYLN-ELEIETMPSPTYMDRQ-KELAW KMRGILTDNLIEVHSRFRLLPETLFLAVNIIDRFLSLRVCSLNKQLVGTAAALF
CLN1-Sc	IELSNAELLTHYETIQEYHEEISQNVL-VQSSKTKPDIKLIDQQPEMNP QTREAIVTFLYQLSVMTVRVSNIGIFFHSVRFYDRYCSKRVVLKDQAKLVVGTCLW
CLN3-Sc	PNLVKRELQAHHSASEYNNDQLDHYF-RLSHTERPLYNL3NSQPQVNP- KMRFLIFDFIMYCHTRLNLSTSTLFTFTILDYSSRFIIKSYNYQLLSLTALW
CYCD1-Hs	VASKMKETIPLTAEKLCIYTDGSIIRPEELLQMELELLVNLKLNLAAMTPH EFIEHFLSKMPEAEENKQIIRKHAQTFVALCATDVKFISNPPSMVAAGSVVAAV (SEQ ID No. 7)
CYCA-Hs	LASKFEEIYPPEVAEFVYITVDITYTKKQVLRMEHLVLKVLTFDLAAPT QFLTQ-YFLHQ2NCKVESLAMFLGELSLLIDAD--PYLKYLPSVIAGAAFHIAL (SEQ ID No. 8)
CYCA-Dm	IAAKYEEIYPPEVGEFVFLTODSYTKAQVLRMEQVILKILSFDLCTPTAY VFINT-YAVLCDMPEKLYMTLYISELSIMEGE--TYLQYLPSLMSSASVALAR (SEQ ID No. 9)

FIGURE 5A

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CYCB1-Hs IASKYEEMYPPEIGDFAFVTDNVTYTKHQIRQMEMKILRALNFGGLRPLPL  
HFLRR-ASKIGEVDVEQHTLAKYLMELTMLDYD---MVHFPSPQIAAGAFCLAL  
(SEQ ID No. 10)

CDC13-Sp IASKYEEVMCPSVQNFVYMADGGYDEEEILQAERYILRVLEFNLAYPNPM  
NFLRR-ISKADFYDIQTRTVAKYLVVEIGLLDHK---LLPYPPSQQCAAAMYLAR  
(SEQ ID No. 11)

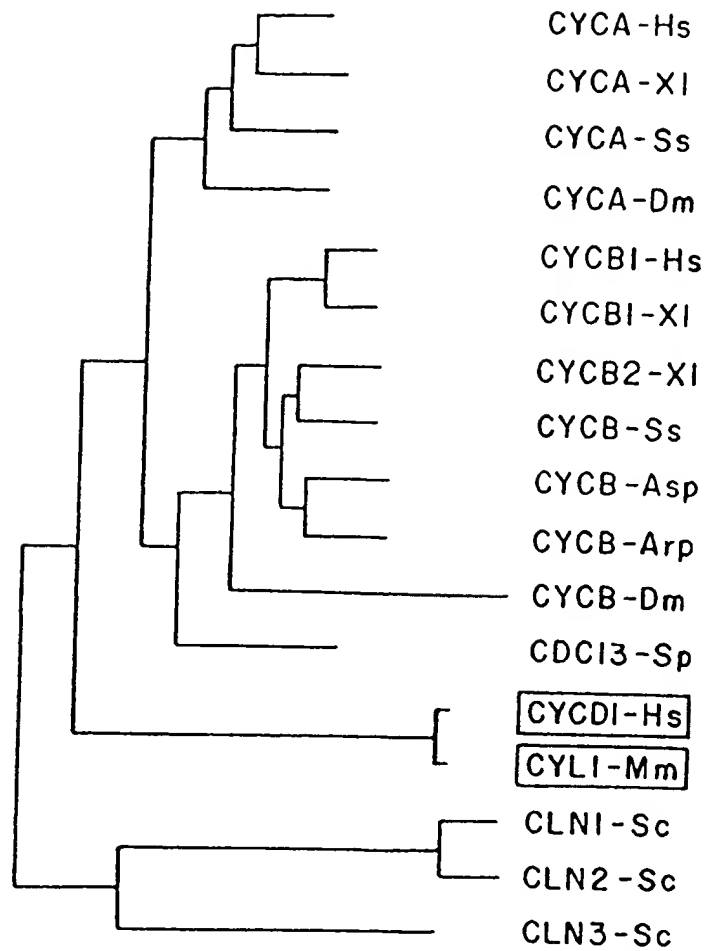
CLN1-Sc LAAKTWG25RLSELVHYCGGSDLFDESMFIQMERHILDTLNWDVVEPMIN  
DYI (SEQ ID No. 12)

CLN3-Sc ISSKFW3RMATLKVQLNLCNQYSIKQFTTMMHFLFKSLDWSI2SATFD  
SYI (SEQ ID No. 13)

FIGURE 5A (cont.)

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FIG. 5B





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CYCD1-Hs	MEHQLLCCEVETI-RRAYPDANLL-NDRVLRAMLKAEETCAPSVSYFKCVQKEVLPS  HCND11 HCND12  MRKIVATWMLLEVCEEOKCEEVFPLAMNYLDRFLSLEPVKKSR
CYL1-Mm	MENQLLCCCEVETI-RRAYPDTNLL-NDRVLRAMLKTEETCAPSVSYFKCVQKEIVPS  MRKIVATWMLLEVCEEOKCEEVFPLAMNYLDRFLSLEPLKKS
CYCD2-Hs	MELLCHEVDPVRRVDRNLLR-DDRVLQNLITIEERYLPQCSYFKCVQKDIQPY  MRRMVATWMLLEVCEEOKCEEVFPLAMNYLDRFLAGVPTPKSH
CYL2-Mm	MRRMVATWMLLEVCEEOKCEEVFPLAMNYLDRFLAGVPTPKTH
CYCD3-Hs	MELLCCGTRHAPRAGDPRLLDQDQVLSLLRLEERYVPRASYFQCVQREIKPH  MRKMLAYWMLLEVCEEOKCEEVFPLAMNYLDRFLSCVPTRKAQ
CYL3-Mm	MRKMLAYWMLLEVCEEOKCEEVFPLAMNYLDRFLSCVPTRKAQ
CYCA-Hs	MRAILLVDWLVEVGEEYKQLQNETLHLAVNYIDRFLSSMSVLRGK
CYCB1-Hs	MRAILLVDWLQVQMKFRLLQETNMTVTSIIDRFMQNNCVPKKM
CYCB2-Hs	MRAILLVDWLQVHSHKFRLLQETLYMCGIMDRFLQVQPVSRKK
CYCC-Hs	LQIFFFTNVIQALGEHLKLRQQVIATATVYFKRFYARYSLKSID
CYCE-Hs	MRAILLDWLMEVCEVYKHLHRETFYLAQDEFDRVMA2ENVVKTLL Cyclin Box

FIG. 7

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HCND13

CYCD1-Hs	<u>LQLLGATCMFVASKMKETIPLTAEKLCIYTDGSI</u> <u>RPEELLMQELLVNKLKWNLAAMTPHDFI</u> EHFLSKMPEAEENKQIRKHAQTFVLCATDVKFIS (SEQ ID No. 25)
CYL1-Mm	<u>LQLLGATCMFVASKMKETIPLTAEKLCIYTDNSIRPEELLMQELLVNKLKWNLAAMTPHDFI</u> EHFLSKMPDAEENKQIRKHAQTFVLCATDVKFIS (SEQ ID No. 26)
CYCD2-Hs	<u>LQLLGAVCMFLASKLKETSPLTAEKLCIYTDNSIKPQELLEWEVLVGLKLNLAAVTPHDFI</u> EHILRKLPQOREKLSLIRKHAQTFVLCATDFKFAMY (SEQ ID No. 27)
CYCL2-Mm	<u>LQLLGAVCMFLASKLKETSPLTAEKLCIYTDNSVVKPQELLEWEVLVGLKLNLAAVTPHDFI</u> EHILRKLPQOREKLSLIRKHAQTFVLCATDFKFAMY (SEQ ID No. 28)
CYCD3-Hs	<u>LQLLGAVCMFLASKLKETSPLTAEKLCIYTDHVSQRQLRDWEVLVGLKLNLAAVIAHDFL</u> AFILHRISLPRDRQALVKKHAQTFVLCATDYTFAMY (SEQ ID No. 29)
CYL3-Mm	<u>LQLLGTVCILLASKLRETTPLTIEKLCIYTDQAVAPWQLREWEVLVGLKLNLAAVIAHDFL</u> ALILHRISLPSDRQALVKKHAQTFVLCATDYTFAMY (SEQ ID No. 30)
CYCA-Hs	<u>LQLVGTAAMLLASKFEEIYPPEVAEFVITDDTYTKQVLRMEHLVLKVLTDFDLAAPTVMNQFL</u> (SEQ ID No. 31)
CYCB1-Hs	<u>LQLVGVTAMFIASKYEEMYPPEIGDAFVTDNTYTKHQIQMEMKILRALNFGGLRPLPLHFL</u> (SEQ ID No. 32)
CYCB2-Hs	<u>LQLVGITALLASKYEEMFSPNIEDFVYITDNTAYTSSQIREMETLILKELKFEGLRPLPLHFL</u> (SEQ ID No. 33)
CYCC-Hs	<u>PVLMAPTCVFLASKVEEI6LKTRFSYAPPKEFPYRMNHILECEFYELLEMDCCCLIVYHPYRPL</u> (SEQ ID No. 34)
CYCE-Hs	<u>LQLIGISSLFIAAKLEEIYPPKLHOFAYVTDGACSGDEILTMELMIMKALKWRLSPLTIVSW</u> (SEQ ID No. 35)

Cyclin Box

FIG. 7 (cont.)

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CYCD1-Hs	PPSMVAAGSVVAAVKGLNLRSPNNFLSYVRLRFLSRVIKCDPDCLRACQ EQIEALLLESSLRQAQQNMDPKA-AEEEEEEEEVDLACTPTDVRDVI* (SEQ ID No. 19)
CYL1-Mm	PPSMVAAGSMVAA MQNLGSPNNFLSRVTRTHFLSRVIKCDPDCLRACQ EQIEALLLESSLRQAQQNMDPKA-TEEEGEVEEEAGLACTPTDVRDVI* (SEQ ID No. 20)
CYCD2-Hs	PPSMIATGSVGAAICGLKQDEEVSSLTCDALTELLAKITNTDVCCLKACQ EQIEAVLLNSLQQYRQDQRD-----GSKSELDQASTPTDVRDIDL* (SEQ ID No. 21)
CYL2-Mm	PPSMIATGSVGAAICGLQDDDEVNTLTCDALTELLAKITHTDVDCCLKACQ EQIEALLNSLQQFRQEQHNA-----GSKSVEDPDQATTPTDVRDIDL* (SEQ ID No. 22)
CYCD3-Hs	PPSMIATGSIGA AVQGLGACS-----MSGDELTELLAGITGTEVDCLRACQ EQIEAALRESLREAAQTSSSPAPKAPRGSSSQGPSQTSTPTDVTAIHL* (SEQ ID No. 23)
CYL3-Mm	PPSMIATGSIGA AVIGLGACS-----MSADELLELLAGITGTEVDCLRACQ EQIEAALRESLREAAQTAPSPVPKAPRGSSSQGPSQTSTPTDVTAIHL* (SEQ ID No. 24)

FIG. 7 (cont.)

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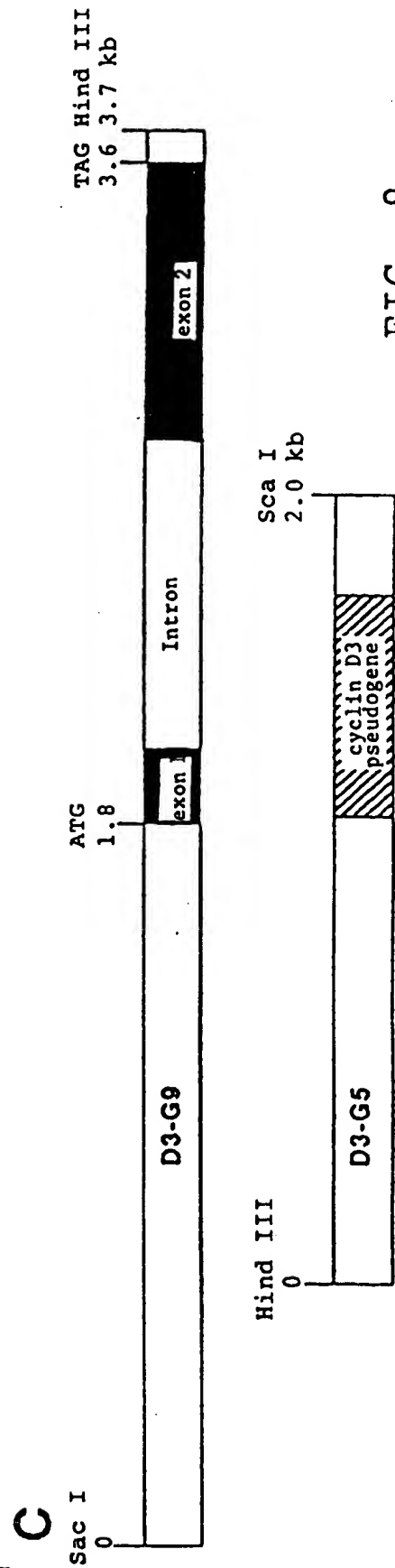
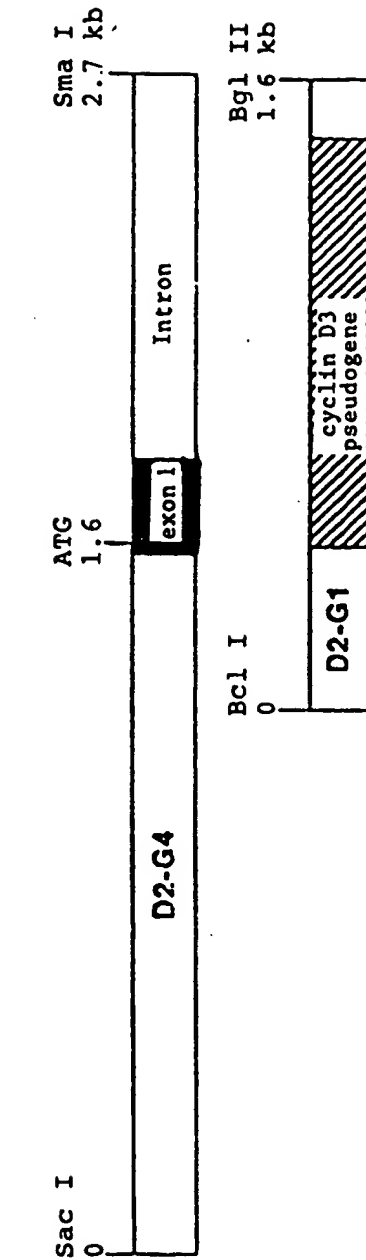
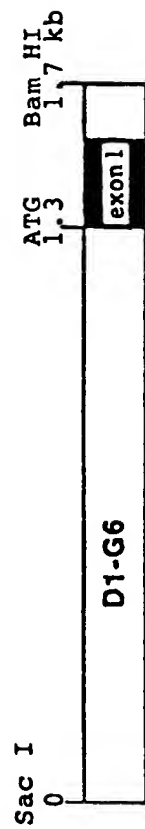


FIG. 8



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TGATCAAGTTGACACTCAATATTAAACCCCTCATAGACTGTGATCCCTATGTTGCTGCCTT 120  
 CCTCGTTTCTATTGCTCTTTGGCCCCCAACCCAAATAAGGTTCTTGGGACACACTAAAGA  
 AGGAGGTGGAGTTCGAAGGGAGGAGAGATGTGAGCGAGGCAGGCAGGGAAGCTCTGCT 240  
 CGCCCACTGCCCAATCCTCACCTCTCTTCTCCTCCACCTTCTGTCTCTGCCCTCACCTCTC  
 CTCTGAAAACCCCCCTATTGAGCCAAAGGAAGGAGATGAGGGGAATGCTTTTGCCCTTCCC 360  
 CCTCCAAAACAAAAACAAAAACAAACACACTTTCCAGTCCAGAGAAAGCAGGGAGTGAG  
 GGTTCACAGAGCTGGCCATGCAGCTGCTGGGCTGTGAGGTAGACCCGGTCTCTCAGAGCC  
 M Q L L G C E V D P V L R A  
 ACGAGGACTGCAACCTACTCCAAGTTGACCGTGTCTCTGAAGAACCTGCTTGCTATCAAGA 480  
 T R D C N L L Q V D R V L K N L L A I K  
 AGCGTACCTTCAGTAATGCTCCTACTTCAAGTGTGTGCAGAAAGGCCATCCAGCCGTAC  
 K R Y L Q \* C S Y F K C V Q K A I Q P Y  
 ATGCACAGGATGGTCCCACTTCTGATGTGTGCCCATTTGATGTGTGCCCACTTCTGATGTTGG 600  
 M H R M V P L L M V [ insertion  
 CCAACATGATTGAACCATTTGGGATGGAAAGCACCTTTACTCTCAGCCACCTGTTAAC  
insertion  
 TAATGCTGGAGGTCTGTGAGGAACAGAAAGTGTGAAGAAAGGTTTTCCTCTGCGCCACGAT 720  
 J M L E V C E E Q K C E E K V F P L A T I  
 TTACCTGGACTGTTTCTTCGCCAGGATCCCAACTTCAAAGTCCCATCTGCAACTCCTGG  
 Y L D C F F A R I P T S K S H L Q L L  
 GTGCTGTCTGCATGTTCCCTGGCCTCCAGGCTCAAAGAGTCCAGCCCACTGACTGCCAAAAA 840  
 G A V C M F L A S R L K E S S P L T A K K

FIGURE 9

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GCTGTGCAATTATACCGACAACTCCATCAAGCCCTCAGGAGCTGCTGGAGTGGAACTGG  
 L C I Y T D N S I K P Q E L L E Q E L  
 TGGTGTGGGAAAGTTGAAGTGGAACTGGCAGCTGTACGCCCTCATGACTTATTAGTA 960  
 V V L G K L K W N L A A V T P H D F I \* Y  
  
 CATCTTGCACAAGCTGCCCCCAGCAGCGGGGAGAAGCTGTCTCCAATCTGCAAGCAAGTCC  
 I L H K L P Q Q R E K L S [ deletion  
 AGAACTTCAATGCTCTGTATGCAATGTACCGCCATCAATGGTTGCAACTGGAAGTGTAGG 1080  
 ] A M Y P P S M V A T G S V G  
  
 AGCAGCTATCTGTGGACTTCAGCAACATGAGGAAGTGAAGTCACTCCCTTGCAATGCCC  
 A A I C G L Q Q H E E V S L P C N A  
 TGA CTGAGCTGCTGGCAAGATCACCACACAGATGTGGATTGTCTCAAAAGCCCAACCGG 1200  
 L T E L L A K I T N T D V D C L K \ A N R  
  
 AGCATATTGAGGTGGTCTTCCTCAACAGCCTGCAGCAGTGCCATCAGGACCAGCAGGAC  
 E H I E V V F L N S L Q Q C H Q D Q Q D  
 AGATCCAAGTCAGAGGATGAAGTGGGCCAAGCAGCACCCCTATAGACCTGTGAGATATCGA 1320  
 R S K S E D E L G Q A \ S T P I D L \* D I D  
  
 CCTGTGAGGATGGCAGTCCAGCTGAGAGGGCATTCATAATCTGTGTCTCCTCTTCTTC  
 L \* (SEQ ID No. 31)  
 TGGTTATGTTTGTCTTGTATCTTAGGGCGAAACTTAAAAAACCCTCTGCCCCCA 1440  
 CATAGTTCGTGTTAAAGATCT 1462 (SEQ ID No. 30)

FIGURE 9 (continued)

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[illegible]

**FIGURE 10**

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AAGGCTGAGGACCCGACAGACCCAGGGGATCAGCGCGTCTGCAGAGCTTGCTCCCT  
E G S R/D P Q T P G D Q R V L Q S L L P  
TGGAGTAGCGCTGCGTGCACCTGCGCCTACTTCCAGTCCGTGCAAGGGAGAGCAAGCCGCA 1320  
L E \* R C V H C A Y F Q C V Q R E S K P H

CATCGGAAGATGCTGGTTTACTGGATGCTGGAGGTGTGTGAGGAGCAGTGTGTGAGG  
M R K M L V Y W M L E V C E E O C C E  
AGGAGCAGTGTGTGTAAGGAGGAAGTCTTCCCTGGCCATGAACCACTGCATGCTACCTG 1440  
E E O C C K E E V F P L A M N H L H A T C

TCCTACGTCCTCCACCCGAAAGGCACAGTTGCAGCTCTTGGTGGGTCTCCATGC  
P T S P P T R K A Q L Q L L V A V S M  
GGCTGGCCTCCAAGCTGCGTAAGACTGGGCCCATGACCATTGAGAAATGTGCATCTACAC 1560  
R L A S K L R K T G P M T I E K M C I Y T

CGACCAGCTGTCTCTCCCTGCCAGTGGCGGAGTGGGAGGTGATGCTCCTGGGAAGC  
D H A V S P C Q L R D W E V M V L G K  
TCAAATGGGACCTGGCCGCTGTGATTGCTCATGACTTCTTGGCCCTCATCTGCACCGACC 1680  
L K W D L A A V I A H D F L A L I L H R \

GACAGGCCTTGGTCAAAAGCATGCCAGATCTTTTGGCTGTCTGTGTACAGATTAC  
R Q A L V K K H A Q I F L A V C A T D Y  
ACCTTTGCCATGTACCCACCATCCAGTTGTGAAAACAAACCAATGCCTGTAACTGATGA 1800  
T F A M Y P P S S C E N N P N A C \*  
(SEQ ID No. 33)

ACAGATAACCATATGTGATATATATCAATAGGAATATGGCCTGGCATGCTGGCTT  
ACGCTGTAATCCTGCACCTTGGGAGGCCAAAGTGGAGGATCACTTGAGCCGAGGAGTCAA 1920

GGCCAGCCTGGGCACAAAGTGAGACTCCTTCTAAAAATAATAATAATAATAATAA  
AAACAATGTAATATTATTCAGCCATAGAAAGGAATAAAGTACT 2021  
(SEQ ID No. 32)

FIGURE 10 (continued)

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GAGCTCGATCAGTACACTCGTTTGTTTAATTGATAATTGTCCTGAATTATGCCGGCTCCT  
GCAGCCCCCTCACGCTCACGAATTCAGTCCCAGGGCAAATTCTAAAGGTGAAGGGACGTC  
TACACCCCCAACAAAACCAATTAGGAACCTTCGGTGGGTCTTGTCCTCAGGCAGAGGGGAC  
TAATATTTCCAGCAATTTAATTTCTTTTTTAATTAAAAAAATGAGTCAGAATGGAGATC  
ACTGTTTCTCAGCTTTCATTTCAGAGGTGTGTTTCTCCCGGTAAATTGCCGGCACGGGA  
AGGGAGGGGGTGCAGTTGGGGACCCCCGCAAGGACCGACTGGTCAAGGTAGGAAGGCAGC  
CCGAAGAGTCTCCAGGCTAGAAGGACAAGATGAAGGAAATGCTGGCCACCATCTTGGGCT  
GCTGCTGGAATTTTCGGGCATTTATTTTATTTTATTTTGTGAGCGAGCGCATGCTAAGCT  
GAAATCCCTTTAACTTTTAGGTTACCCCTTGGGCATTTGCAACGACGCCCCGTGTCGCCG  
GAATGAAACTTGACAGGGGTGTGTGCCCGGTCCTCCCCGTCTTGCATGCTAAATTAG  
TTCTTGCAATTTACACGTGTTAATGAAATGAAAGAAGATGCAGTCGCTGAGATTCTTTG  
GCCGTCTGTCCGCCCCGTGGGTGCCCTCGTGGCGTTCTTGGAATGCGCCCATCTGCCCCG  
CTTGATATGGGGTGTGCGCGCGCCCCAGTCACCCCTTCTCGTGGTCTCCCCAGGCTGCG  
TGCTGGCCGGCCTTCCTAGTTGTCCCCTACTGCAGAGCCACCTCCACCTCACCCCTAAA  
TCCCGGGACCCACTCGAGGCGGACGGGCCCCCTGCACCCCTCTCGGCGGGGAGAAAGGCT  
GCAGCGGGGCGATTTGCATTTCTATGAAAACCGGACTACAGGGGCAACTGCCCGCAGGGC  
AGCGCGGCGCCTCAGGGATGGCTTTTCGTCTGCCCCCTCGCTGCTCCCGGCGTTCTGCCCCG  
CGCCCCCTCCCCCTGCGCCCCGCCCCGCCCCCTCCCGCTCCCATCTCTGCCGGGCTTT  
GATCTTTGCTTAACAACAGTAACGTACACGGACTACAGGGGAGTTTTGTTGAAGTTGCA  
AAGTCCTGGAGCCTCCAGAGGGCTGTGGCGCAGTAGCAGCGAGCAGCAGAGTCCGCACG  
CTCCGGCGAGGGGCAGAAGAGCGCGAGGGAGCGCGGGGCAGCAGAAGCGAGAGCCGAGCG  
CGGACCCAGCCAGGACCCACAGCCCTCCCCAGCTGCCCAGGAAGAGCCCCAGCCATG  
(SEQ ID No. 34)

FIGURE 11

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GAGCTCGAGCCACGCCATGCCCCGCTGCACGTGCCAGCTTGGCCAGCACATCAGGGCGCTG  
GTCTCTCCCCTTCCTCCTGGAGTGAAATACACCAAAGGGCGCGGTGGGGGTGGGGGTGA  
CGGGAGGAAGGAGGTGAAGAAACGCCACCAGATCGTATCTCCTGTAAAGACAGCCTTGAC  
TCAAGGATGCGTTAGAGCACGTGTGAGGGCCGACCGTGCTGGCGGCGACTTCACCCGAGT  
CGGCTCCCAGGGAGAAAGCCTGGCGAGTGAGGCGCGAAACCGGAGGGGTGCGCGAGGATG  
CGGGCGAAGGACCGAGCGTGGAGGCCTCATGCTCCGGGAAAGGAAGGGGTGGTGGTGT  
TGCGCAGGGGGAGCGAGGGGGAGCCGGACCTAATCCCTTCACTCGCCCCCTTCCCTCCCG  
GGCCATTTCTAGAAAGCTGCATCGGTGTGGCCACGCTCAGCGCAGACACCTCGGGCGGC  
TTGTCAGCAGATGCAGGGGGGAGGAAGCGGGTTTTTCTGCGTGGCCGCTGGCGCGGGGG  
AACCGCTGGGAGCCCTGCCCCCGGCTGCGGCGGGCCCTAGACGCTGCACCGCGTCGCCCC  
ACGGCGCCCCGAAGAGCCCCCAGAAACACGATGGTTTTCTGCTCGAGGATCACATTCTATCC  
CTCCAGAGAAGCACCCCCCTTCTTCCTAATACCCACCTCTCCCTCCCTCTTCTTCTCT  
GCACACACTCTGCAGGGGGGGGAGGAAGGGACGTTGTTCTGGTCCCTTTAATCGGGGCTT  
TCGAAACAGCTTCGAAGTTATCAGGAACACAGACTTCAGGGACATGACCTTTATCTCTGG  
GTATGCGAGGTTGCTATTTTCTAAATCACCCCTCCCTTATTTTTCACTTAAGGGACCT  
ATTTCTAAATTGTCTGAGGTCACCCCATCTTCAGATAATCTACCCTACATTCCTGGATCT  
TAAATACAAGGGCAGGAGGATTAGGATCCGTTTTTGAAGAAGCCAAAGTTGGAGGGTCTG  
ATTTTGGCGTGCTACACCTACAGAATGAGTGAAATTAGAGGGCAGAAATAGGAGTCGGTA  
GTTTTTGTGGGTGCGCCTGTCCGGGCCCCCTGGCATGCAGGCTTGGATGGAGGGAGAGGG  
GTTGGGGGTTGCGGGGGACCGCGTTTGAAGTTGGGTGCGGGCCAGCTGCTGTTCTCCTTAA  
TAACGAGAGGGGAAAAGGAGGGAGGGAGGGAGAGATTGAAAGGAGGAGGGGAGGACCGGG  
AGGGGAGGAAAGGGGAGGAGGAACCAGAGCGGGGAGCGCGGGGAGAGGGAGGAGAGCTAA  
CTGCCCAGCCAGCTTCGGTCACGCTTCAGAGCGGAGAAGAGCGAGCAGGGGAGAGCGAGA  
CCAGTTTTTAAGGGGAGGACCGGTGCGAGTGAGGCAGCCCCTAGGCTCTGCTCGCCCCACCA  
CCCAATCCTCGCCTCCCTTCTGCTCCACCTTCTCTCTGCCCCACCTCTCCCCCGAAA  
ACCCCTATTTAGCCAAAGGAAGGAGGTCAGGGAACGCTCTCCCCTCCCTTCCAAAAAA  
CAAAAACAGAAAAACCCTTTTCCAGGCCGGGGAAAGCAGGAGGGAGAGGGCGCGGGCTGC  
CATG (SEQ ID No. 35)

FIGURE 12

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GAGCTCCCGTCCCCATACTACAGGTTACATCCAGCTTTCAGGACTAGTCAGTCTATGTG  
GCCCTCCCTCAATTAATAAATCAGCACTAATTTGCCAGGTGCGGTGGTTTGTGCCTGTA  
ATCCCAGCACTTTAGGAAGCTGAGGCAGGCAGATCACTTGAGGTCAGGAGTTCGAGACCA  
GCCTGGCCAAACATGGTGAAATCCCGTATCTACTGAAAATACAAAAATTAGCCGGGCATGG  
TGGTATGCACCCGTAATCCCAGCTACTCAGGAAGCTGAGGCAGGAGAATCACTTGAAACC  
GGGAGGCAGAGGTTGCAGTAAGCTGCACTCCAGCCTGGTGACAAGAGCAAAACTTTGTGT  
CAAAAAACAAAGAAAACCAAAAAACAAAGGAAAACACAAAAACCCCTTCTATTTGTAA  
AAAAAATAATCCACCGTGAACCAAAAAATTAGTAAAAACAATGAACTAAAATTTTGT  
TTGCAAAATGTATGATAACAAATGTTAAGGAAGGTCATGTGCCGTTATGGTTCACTGCA  
GCCTTGAACCTCCTGGGCTCAAGCGATCCTCCTGCTTCGGTCTCCCTAGTAGCTGGGACTA  
CAGGCTTGTGCCACCGCACCCAGCTTATTTTTTTTTTTTATTTTTTGTAGAGATAGGAGT  
CTTGCTTTGTGTGCCAGGCTGGTCTTCAACTCCTAGCTTCCAGTGATCCTCCTGCCTCAG  
CCTCCCAAGTGCTGGGCTGATGGGACATTTTATACATAGTGCCATGTACCTATAAATG  
AGAAGTTTTAAAAATACTGATTTTTAAAAATTAATTTATGTCAAGAATTTTATACCAAAG  
TAAAAAACCAACCGAAAATATGAAAAGGGTTAATATCTTTGAGAGGTGATGAGAAGT  
ATAAGTCAATAAGAGAAAAACAACATCCCTATAAATGAATAAGCTAAGGACATGAATGGG  
TAATGTACATAAGAAATGTAAATGTCTAGTAATATGCCAAAATAGATTTATTATTACTAA  
TAAGCCACTTTCACTCTCTAGTTGGCAGAGTTGTTTTGAAAAATAGATATGTAATGATGG  
TGGAAAAGATTGGTTTAACTATTCAGCAGGAAAATTTGGCAATTAGAAGTGTATCAAAAG  
CCTTAGAATGTTTCATAACCTTAGATTGGGAAATTCCACTTCTAGAAATTAATTCACCTC  
TAGAAATAATCATGAGTGTGCACAAAGATATTACCACAAAAATATTTTACAGTATTATGT  
CTAATAGAGAAGAACTAGAAAATAATTTAAATTTCCACCAATACAGGTTTGCCAAAATACA  
TTTTGTACATTACCTAATGGTATATTATGTCCCTATTACAAATACGTCCTAGAATATT  
TAATAGCATGGAAAAGTGTTAACAGTATTTTTTTAATGAAAAAGCTTACAAAACAGTTT  
GTGATGATTCCATTAAAAATGTGTGTTTATTATAGAACAAAGATTAGAAAAATAACAT  
TGATATATTAAAGGGTTATTTTCATGGCAAATGCAAATGATTATTTCCCTTTTTTGTGGC  
TTATTTGTATTTTTTGAAGTTTTCTACAATGTAAAAGAATATTTTATGATAGAAAACAT  
AATACAATTTATAATATAAGAAAGAATAATTCGGCCGGGAACGGTGGCTCAGCCTGTAA  
TCCCAGCACTTTTGGAGGCCGAGACCGGCGGATCACGAGGTGAGGGGTTCAAGACTAGCC  
TGGCCAACATAGTGAAACCCCATCTCTACGAAAAATACAAAAATTAGTCAGGCATGGTGG  
TGCGTGCCTGTAGTCCCAGCTACTCGGGAATTGCTTGAACCCGGGAGGTGGAGGTTGCAG  
TGAGCCCAGATCGCACCCTGCACTCCAGCTTGAGCAACAGAGTAGACTTCGTCTCAAAA  
AAAAAAAAAAAAAAAAAAGAATAATTAACAGAAAATGGTTAGACACTTCCTTAGTGTCT  
CCTAAGTCAGGAGGACCCAGTAGGGCAGGGATCCTCATGGCCTCCTCCCATTTGGAGCA  
TTATTGGAGGTCTTTTTTCGGCCTCTTCGTCAAGTGGAATCTAGCTTCCGGTAAAACCTACA  
AAGTAACCAAAAGTTTGGGAGGTGGAAGAAATGCAACCGGTAGATCTCACAGAGTCTGTG  
CAAGAACTGATTCAATGAGAATCTAGTTTCTCCGTCCACAGTTTCTCCAAACAGAACT  
AAGGCCGACTTTAGGGGCTTGTCCAAACCTAGGCAAGCAACTTAACAAGGTGAGGCCATG  
ACTCCATGGCCTTTCCGTTCTGTTATATGCTGACTTAGACTAAAGCTCTCATACTTTAAA  
GTGCACAGAAATCTAGTTAAAATGCAGATTCTGATTCAGGTTAGGGGTGGGCCTGAGAGT  
CTGCATTTCTAACCCAGCTCCCAGCGATGACCAACGACGGGACAGGCTGGGATCACAGT  
TTAACTAGCAATGGTGTAGAACACAGAATCTGCAGCAAGAAGGCCAGCTTCCCAATCCTA  
GCTCTGCCACGGACCAACTGAATGACAGTTGCCTCGGTTTCCGAGTTTTCTGTGAAGATGT  
AGTGAGTCATTACATCGTGAGGCTTTCGAGCAGCGTTCACCTAAGAAGTCTGACATT  
ATTTATCGCATTCCTTAGAGCAAGCAGCCGGTGAAGTAGGGTTTGACGAATGAATAAGTG  
AATGAATGACCTTTGGAGAAAAATTGTTTCTGGGTGACTAGAGTCCGAGAAGCAAAATG  
GGAGGGCCCGTGGTGGGTAGGAGGCCACCTCCTAGAAAGTTCTCTGCACCCGGTGGTCC  
AGAGGGCCTGGAGTGCCGGAAGCCGGCCGCGTTGCGCTCACGGCCCAATGGGGCCGCGGG  
AGGGAGGGGAGAGCGCTCAGCCAACCTTTCCGTTCCGGGCGCCGACGCCCCGCCCCCTCG  
GAGCGTTGCGACGTCCGAGCATTCCACGGTTGCTACATCGTCCGAGGGGGGGCGCCTGT  
CAGGGAAGCGGCGCGCGCGGGCGGGCGGGCTGGGGATCCGCGCGCAGTGCCAGC  
GCCAGCGCCAGACCCGCGCCCCGCGCTCTCCGGCCCCGTCGCTGTCTTGGGACTCGCGAG  
CCCGCACTCCCGCCCTGCCTGTTGCTGCCCGAGTATG (SEQ ID No. 36)

FIGURE 13  
SUBSTITUTE SHEET

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/05000

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07H 21/04, C07K 13/00

US CL : 530/350, 536/23.1, 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 536/23.1, 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, APS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Oncogene, Volume 6, No. 3, issued March 1991, Rosenberg et al., "Rearrangement and overexpression of D11S287E, a candidate oncogene on chromosome 11q13 in benign parathyroid tumors," p. 449-453, see entire document.	1-36
Y	Nature, Volume 350, issued 11 April 1991, Motokura et al., "A novel cyclin encoded by a bcl1-linked candidate oncogene," p. 512-515, see entire document.	1-36

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

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Date of the actual completion of the international search

12 July 1993

Date of mailing of the international search report

05 AUG 1993

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